# MORPHOLOGICAL AND GENETIC COMPARISONS BETWEEN *BABESIA BOVIS* AND *TRYPANOSOMA* SPP. FOUND IN CATTLE AND WHITE-TAILED DEER

A Thesis

by

AMANDA CHRISTINA FISHER

Submitted to the Office of Graduate Studies of
Texas A&M University
in partial fulfillment of the requirements for the degree of

MASTER OF SCIENCE

August 2012

Major Subject: Laboratory Animal Medicine

Morphological and Genetic Comparisons between *Babesia bovis* and *Trypanosoma* spp.

Found in Cattle and White-tailed Deer

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Approved by:

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# ABSTRACT

Morphological and Genetic Comparisons between *Babesia bovis* and *Trypanosoma* spp.

Found in Cattle and White-tailed Deer.

(August 2012)

Amanda Christina Fisher, B.A., Hartwick College; DVM, Atlantic Veterinary College

Co-Chairs of Advisory Committee: Dr. Patricia Holman
Dr. Donald Davis

*Babesia bovis* has been an important disease agent in the U.S. cattle industry for over a century. Recently, *B. bovis*-like parasites have been identified in white-tailed deer (WTD; *Odocoileus virginianus*) in Texas. If the parasites found in the WTD are *B. bovis* that are able to infect cattle, the disease could re-emerge. Susceptible adult cattle often die from this disease, which would result in severe production losses, as well as a decrease in carcass weights of disease survivors. The *B. bovis*-like parasite found in WTD was compared to *B. bovis* from cattle, by ribosomal DNA sequence analysis. *Babesia* isolated from WTD were found to have 99% identity to *B. bovis* from GenBank cattle sequences. No cattle samples in this study were found to be positive for *B. bovis*. On culture of WTD samples, a *Babesia* parasite could not be visualized based on common morphological features.

*Trypanosoma cervi* has been studied for decades, but all the previous research identified this parasite solely by morphology. *Trypanosoma* species obtained from different host species was compared by ribosomal DNA sequence analyses. In this study, the *Trypanosoma* cultured from WTD had the morphological appearance of *T. cervi.* On sequence analysis, the cattle sequences aligned together with cattle isolates and the WTD sequences aligned closely with elk (*Cervus canadensis*) sequences, indicating that wild ungulates (WTD and elk) and cattle most likely have separate trypanosome species. On distribution analysis there was a trend in three South Texas counties, where the county with the highest occurrence of *Trypanosoma* had the lowest occurrence of *Babesia*; and vice versa. It is possible that *Trypanosoma* and *Babesia* blood parasites compete within the mammalian host, but the $\chi^2$ test did not show a significant association between the two parasites in the different counties. On seasonal analysis, the correlation between positive samples and season could not be statistically confirmed, but it appears that *Babesia* infected animals are found in lowest numbers during hot, dry seasons. It also appears that there is another vector for *Trypanosoma* in South Texas besides the ked (*Lipoptena mazamae*) and tabanid fly (*Tabanus* spp.).

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I would like to thank my committee chair, Dr. Holman, for all her support and long hours helping me to make this happen. I would like to thank my committee members, Dr. Davis and Dr. Gresham, for their guidance and support throughout the course of this research and during my residency program.

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Finally, thanks to my mother and father for their encouragement.

# NOMENCLATURE

| | |
|---|---|
| PCR | polymerase chain reaction |
| IFAT | indirect immunofluorescence antibody test |
| RNA | ribonucleic acid |
| ITS | internal transcribed spacer |
| DNA | deoxyribonucleic acid |
| SSU rRNA | small subunit ribosomal RNA |
| rDNA | ribosomal DNA |
| EDTA | ethylenediamine-tetraacetic acid |
| TBE | tris/Borate/EDTA |
| NCBI | National Center for Biotechnology Information |
| BLAST | Basic Local Alignment Search Tool |
| FBS | fetal bovine serum |
| $\chi^2$ | Chi squared test |
| $\gamma\delta$ | Gamma Delta T cells |
| INF-$\gamma$ | Interferon Gamma |
| PAUP | Phylogenetic Analysis Using Parsimony |

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# CHAPTER I

# *BABESIA* AND *TRYPANOSOMA*: AN INTRODUCTION

## *BABESIA BOVIS*

### Background

The genus *Babesia* is in the family Babesiidae, order Eucoccidiorida, suborder Piroplasmorina, class Sporozoasida, and phylum Apicomplexa (Allsopp et al., 1994). *Babesia* is a relatively small intraerythrocytic protozoan parasite measuring approximately 2 x 1.5 μm (Potgieter and Els, 1979; Ristic and Kreier, 1981). They can be visualized microscopically in blood smears using Giemsa or Romanowsky stains and can be elliptical, spherical, or ring shaped. They are classically found in pairs since during trophozoite multiplication, the formed merozoites remain joined at their posterior poles (Potgieter and Els, 1979). They can also sometimes be seen as singular piroplasms within the red blood cell (Smeal, 1995). The parasite body has a pellicular complex that consists of three membranes (Mehlhorn and Schein, 1984; Todorovic et al., 1981). One nucleus and one nucleolus with aggregated nuclear material surrounded by two nuclear membranes are present (Todorovic et al., 1981). The parasite organelles include polar rings, rhoptries, spherical bodies, mitochondria-like structures, Maurer's

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This thesis follows the style of Veterinary Parasitology.

clefts and spheroid bodies (the function of which is unknown) close to the nucleus (Mehlhorn and Schein, 1984; Todorovic et al., 1981).

*Babesia bovis* was first described by Babes (1888) in Romania and associated with red water fever in cattle (Uilenberg, 2006). He believed it to be a bacterium which he named *Haematococcus bovis* (Bock et al., 2004). It was later renamed *Babesia bovis* (Angus, 1996; Uilenberg, 2006). Smith and Kilborne (1893) were the first to demonstrate that the parasite was transmitted by a tick, *Rhipicephalus (Boophilus) annulatus* (Bock et al., 2004; Graham and Hourrigan, 1977; Uilenberg, 2006).

The cattle fever ticks *Rhipicephalus (Boophilus) annulatus* and *Rhipicephalus (Boophilus) microplus* were originally called *Boophilus annulatus* and *Boophilus microplus* and were eradicated from the United States in 1943 with a program that began in 1907 (Graham and Hourrigan, 1977; Howell et al., 2007). Tick populations are dependent on temperature and relative humidity in different habitats (Corson et al., 2004) and the program was designed to dip livestock carriers before the tick could complete its life cycle and at the same time set up tick inspectors to man the campaign (Graham and Hourrigan, 1977). The program also employed pasture vacation to decrease the survival of unfed larvae in pastures. At the same time it was found that in southern states the tick survived much longer in the winter than summer probably due to summer desiccation (Graham and Hourrigan, 1977). Early on, hand picking adult ticks was recommended along with application of lyme-sulfur, nicotine solutions, glycerine, sodium sulfphite, and cresol solutions. In 1903, crude petroleum became the dip of choice. Later on, arsenic at 21 day dipping intervals was recommended. In 1889, Texas' King Ranch constructed the first dipping vat. Chemical testing for use in dipping vats was mostly done at Texas A&M University (Graham and Hourrigan, 1977).

After the eradication of the fever tick, isolated infestations were restricted and regulated with quarantine and chlorinated hydrocarbon or organophosphorus insecticides. Organophosphates were effective, stable, and less likely to produce tissue residues in market carcasses (Graham and Hourrigan, 1977). The use of the insect growth inhibitor spinosad for control of *R. annulatus* was investigated by Davey et al. (2005) but did not prove to be a viable option for control.

Tick vector eradication would be a permanent solution, but is rarely practical, sustainable or justifiable economically (Bock et al., 2004). Tick eradication worked in the United States due to a vigorous program that broke up the tick's life cycle making it unable to reproduce effectively. A buffer zone of quarantined area, 800 km long and 0.4 to 16 km wide, is still maintained in Texas along the Rio Grande from Brownsville to Del Rio (Graham and Hourrigan, 1977). *R. microplus* is encountered on stray Mexican cattle found in Starr, Hidalgo, and Cameron counties in Southern Texas. Yet, endemic stability, or the idea that low level parasitism will create resistant animals (Combrink et al., 2010), is rarely a good disease control strategy (Bock et al., 2004). Bock et al. (2004) makes the point that during a dry season, ticks can be drastically reduced and in turn parasite transmission is reduced, leaving a generation of susceptible animals.

**Life Cycle**

*Babesia* has a two host life cycle with three phases including merogony (asexual reproduction) in the vertebrate host, gamogony in the gut of the tick host, and syngamy (asexual reproduction or sporogony) in the tick salivary glands. The arthropod host, *R. microplus* tick, injects the infective stage, sporozoites into the mammalian host. The tick accomplishes this by secreting salivary fluid and excess water into the vertebrate host in order to concentrate its blood meal, effectively passing the parasite to the vertebrate (Sauer et al., 1995).

The sporozoite actively penetrates the erythrocyte in five phases beginning with the sporozoite aligning with the erythrocyte. The apical pole comes in contact with the erythrocyte and infiltrates the cell with the aid of a specialized apical complex (Potgieter and Els, 1979). Once within the host cell, the sporozoite transforms into a trophozoite, replicates, and forms haploid merozoites (Freeman et al., 2010). To leave the cell, the merozoites rupture the host membrane and escape (Ristic and Kreier, 1981). The parasite can then enter a new red blood cell and undergo replication or form a gamont (Mehlhorn and Schein, 1984)

The adult female *R. microplus* tick then ingests the parasite during feeding (Howell et al., 2007; Mehlhorn and Schein, 1984). Within the tick gut, the gamont escapes the red blood cell and invades tick epithelial cells (Radunz, 2008). Here, gametogenesis and zygote development occur forming kinetes (Howell et al., 2007). The kinete stage enters the tick hemolymph and invades other tick tissues, including the ovarian cells and oocytes. The kinete travels to the developing tick salivary glands and remains dormant until the tick hatches and attaches to a suitable host (Ristic and Kreier, 1981).

Body heat and proximity to warm blooded host are the main stimuli for dormant parasites to reactivate and reproduce by sporogony (i.e. asexual reproduction) (Radunz, 2008). Sporogony occurs in three phases where the kinete nucleus enlarges and becomes lobulated. There is then a division of the cytoplasm by endoplasmic reticulum and invagination of the cell boundary. Sporogony is then completed with differentiation into two new kinetes. The parasite is then able to enter into the vertebrate host and begin the cycle again.

*R. microplus* and *R. annulatus* ticks are one host ticks with larval, nymphal and adult development occurring on one host, preferably cattle (Howell et al., 2007). *Babesia* levels in the adult female tick are not correlated to the parasite levels transovarially passed to their offspring. Howell et al. (2007) found that females with undetectable kinete loads in their hemolymph were still able to pass the parasite to their larval offspring. The larval stage is the only stage of the tick life cycle that can infect a host with *Babesia* (Smeal, 1995) and the parasite does not persist beyond this stage in the tick's life cycle (Mahoney and Mirre, 1979).

**Pathogenicity and Epidemiology**

Severe babesiosis generally occurs in animals over 3-9 months of age. Researchers have also reported that animals infected as calves have a higher resistance to infection as adults (Graham and Hourrigan, 1977; Trueman and Blight, 1978). Age-related resistance is in part from passive immunity received from the dam, but not solely from maternal antibodies (Brown et al., 2006; Uilenberg, 2006). The spleen plays a major role in age-related immunity. Goff et al. (2001) found that inducible nitric oxide synthase was detectible in the spleens of calves and not detected in adults. Another possibility is the abundance of $\gamma\delta$ T cells or a decrease in pro-inflammatory response (Brown et al., 2006). Upon infection, IFN-$\gamma$ levels reach maximal levels in calves in half the time of adults (Brown et al., 2006). Older animals that are infected and survive the infection can also become carriers regardless of the age when infection was acquired (Trueman and Blight, 1978). Carriers are considered the main reservoir for *Babesia*. A high tick infestation is a predisposing factor for clinical disease.

*Bos indicus* cattle are more resistant to babesiosis than purebred British breeds (Callow, 1977). *Bos indicus* are also more resistant than *Bos taurus* and *Bos indicus* crosses (Bock et al., 1997). Corson's model found that fewer larvae survived on *Bos indicus* cattle than on *Bos taurus* (Corson et al., 2004). This may be because *Bos indicus* breeds tend to be from *Babesia* endemic areas which may lead to a natural resistance (Bock et al., 2004).

*Babesia bovis* was found by Callow et al. (1979) to increase in virulence with passaging through spleen intact hosts. Aikawa et al. (1985) found that *B. bovis* produces morphological alterations on the erythrocyte membrane in the form of protrusions that may play a part in the parasite virulence. Immune responses are considered to be in reaction to the *B. bovis* merozoite life cycle stage (Brown et al., 1991). IgM and IgG1 are important in the initial immune response to the parasite (Goff et al., 1982). Adaptive immunity is based on antigen-specific CD4+ T cells through the production of IFN-$\gamma$ (Brown et al., 2006). Macrophages secrete products important for innate and acquired immunity, which include nitrogen oxide, IL-12, TNF-$\alpha$, and IL-18 (Brown et al., 2006; Wright et al., 1989).

Clinical babesiosis is generally seen in adult cattle over one year of age. Depending on virulence and strain, the syndrome can be complicated by vascular derangements (Callow et al., 1979). Early signs in the disease process are a high fever (as high as 42 °C), inappetence and increased thirst. The fever can last 7-10 days and is accompanied by severe depression, reluctance to walk, muscle tremor, and dyspnea. Mahoney et al. (1979) found that the rate of initial parasite removal was greater than parasite replication, which most likely means that erythrocytes were the target for immune attack and destruction. This, along with erythrocyte destruction caused by the parasite itself leads to anemia, jaundice, and hemoglobinuria. Infected erythrocytes are sequestered to microcapillary endothelia leading to hypotensive shock syndrome (Brown et al., 2006). When lung capillaries are infiltrated with parasitized erythrocytes and macrophages, respiratory distress syndrome and death occur (Brown et al., 1991; Wright et al., 1989). Acute forms of the disease can lead to death within 1-2 weeks of onset of fever, while milder, more chronic cases may lead to recovery (Bock et al., 2004). When parasitized erythrocytes infiltrate the brain, the cerebral form ensues (Wright et al., 1989). The cerebral form of the disease can lead to ataxia, circling, head pressing, convulsions, and a collapse into a comatose state. Signs of cerebral damage may be caused by the tendency for parasitized erythrocytes to clump due to erythrocyte membrane abnormality, blocking small capillaries of the brain (Ristic and Kreier, 1981).

Post mortem findings vary between animals. Acute cases generally have anemia, generalized jaundice, ecchymotic hemorrhages on the heart and intestine. More chronic cases can also have an enlarged liver, pulpy spleen, thick granular bile in the gall bladder, congested kidneys and hemoglobinuria (Bock et al., 2004).

**Diagnosis and Treatment**

Clinical signs of persistent fever, jaundice, and hemoglobinuria are suggestive of a diagnosis of babesiosis. Other signs that are indicative of babesiosis are death of several animals in a herd over a period of a few days, necropsy findings, epidemiology of the area or the area of cattle origination, and infestation of the *B. microplus* tick. Blood smears demonstrating the parasite are diagnostic. Since *Babesia* tends to congregate in the smaller capillaries as demonstrated by O’Connor et al. (1999), blood samples from extremities, such as tail or ear margins, should be taken and thick and thin smears should be prepared (Bock et al., 2004). Thin smears are best for detecting acute infection since there will be a high parasite load and thick smears are better for detecting a lower parasitemia, which is indicative of either an animal recovering from the disease or very early infection. Post mortem samples should include impression smears from the kidney and grey matter of the brain. *Babesia* found in the brain should be interpreted with caution as *Babesia* has been found in the brains of clinically normal animals (Smeal, 1995).

Treatment of acute cases includes supportive care and imidocarb dipropionate or diminazene aceturate therapy (Bock et al., 2004). Diminazene works rapidly and is well tolerated (Bock et al., 2004). Imidocarb is a relatively safe drug, but is not labeled for use in cattle in the U.S. Imidocarb has residual effects for up to 4 weeks and severely affected cattle should receive a second dose 24 hours after the first. During a confirmed outbreak all animals showing signs should be given imidocarb and all cattle should be dipped with an effective acaricide. In endemic areas of the world where vaccination is available, vaccination is recommended at 9 months of age. Vaccination should be performed in clinical animals 4 weeks after treatment completion and is 93% effective (Ojeda et al., 2010).

**Summary**

*Babesia bovis* is an important parasite in cattle production in many areas of the world. Based on the parasite life cycle and its effects on susceptible cattle, any reintroduction of the disease to the U.S. could devastate the cattle industry. Babesiosis infection causes large production losses and has been found to decrease carcass weight of surviving market cattle. It has been speculated that WTD could be an important reservoir for this parasite (Kuttler et al., 1972; Cantu et al., 2007; Holman et al., 2010). Kuttler et al. (1972) attempted to establish babesiosis in WTD but was unsuccessful. Their inoculum source was Mexican cattle that were presumed to have *B. bigemina* and *B. argentina* (Kuttler et al., 1972). On the other hand, Cantu et al. (2007) found evidence of the presence of *B. bovis* in a population of WTD in Northern Mexico by nested polymerase chain reaction (nested PCR) and indirect immunofluorescence antibody test (IFAT). Holman et al. (2010) found three *B. bovis* PCR positive free-ranging WTD in Tom Green County, Texas. These isolates were found to have a 97-99% sequence identity to bovine *B. bovis* at the ribosomal RNA internal transcribed spacer (ITS) genomic region and phylogenetically no distinction was evident. Ramos et al. (2010) found *B. bovis* positive samples from free-ranging WTD in southern Texas with 99% sequence identity to the 18S ribosomal DNA to *B. bovis* from cattle.

Tick eradication programs have not worked in the U.S. Virgin Islands, South America or Australia (Graham and Hourrigan, 1977). During the original U.S. Cattle Fever Tick Eradication Program (1906-1943) it was found that WTD populations were very low (Pound et al., 2010). Nevertheless, the regional failures in Florida and Texas during the program in the 1930's have been attributed by some researchers to the small population of WTD present. After tick treatment of WTD, tick eradication was reestablished in Florida and Texas (Pound et al., 2010). Large portions of southern Texas have habitat suitable for fever ticks and WTD (Pound et al., 2010) and WTD populations have increased since 1943. The risk of re-emergence of *Rhipicephalus (Boophilus)* ticks and even babesiosis in the United States is real. *Babesia bovis* has not been eradicated in Mexico, which means that animals, particularly WTD, can cross from Mexico into the U.S. and spread disease.

## ***TRYPANOSOMA* SPP.**

### **Background**

*Trypanosoma* was first named in 1843 by Gruby after his discovery of a frog haemoflagellate, which he named *Trypanosoma sanguinis* (Hoare, 1972). Due to the lifecycle phases that trypanosomes pass through, it was difficult for early scientists to differentiate between distinct species and the different stage parasites. Laveran (1904) was the first to divide the trypanosomes into two groups based on pathogenicity (reviewed by Hoare, 1972). These groups were further subdivided into minor groupings. In 1926 Wenyon established two groupings based on whether the vector stage terminates in the hindgut (stercoraria) or salivary glands (salivaria) of the intermediate invertebrate host. Eventually, the classification based purely on morphological considerations was developed and widely accepted. Since this time, minor alterations and classification revisions have been made as new information is gleaned (Hoare, 1972). Before 1975, trypanosomes of WTD, mule deer (*Odocoileus* *hemionus*), and elk (*Cervus canadensis*) were classified as *Trypanosoma theileri*. In 1975, Kingston and Morton classified trypomastigotes from elk and mule deer as *Trypanosoma cervi* based on morphological and biological distinctions (Matthews et al., 1977).

*T. theileri* occurs most commonly in the blood of cattle (Levine, 1985). It has a long, pointed posterior end with a medium-sized kinetoplast anterior to it. It has a prominent undulating membrane and a free flagellum. *T. cervi* was first distinguished in WTD by Kingston and Morton in 1975. This is a long trypanosome with a short free flagellum and an undulating membrane arising near the kinetoplast (Kingston and Morton, 1975b). The nucleus is large and granular located posteriorly. The kinetoplast is large and usually marginal and posterior to nucleus.

**Life Cycle**

Trypanosomes are a two-host parasite with a life cycle consisting of a vertebrate animal and an invertebrate. The invertebrate introduces the metatrypanosome to the mammal by inoculation during a blood meal (Hoare, 1972). Once in the mammalian host's bloodstream, the metatrypanosome develops into a trypomastigote. The stercorarian trypanosome multiplies discontinuously by binary division in the host bloodstream. Amastigote stages have been seen in the tissues of mammalian hosts, but multiplication occurs within the bloodstream. The trypomastigotes are then picked up by the insect vector during feeding and enter the gut of the insect. Here they pass through the epimastigote form before traveling to the insect's mouthparts and transforming into the metatrypanosome.

**Transmission and Epidemiology**

In Germany, Boese and Petersen (1991) found megatrypanum trypanosomes in the hindgut of Hippoboscids (commonly known as keds) *Lipoptena cervi*. These insects had been collected from red deer (*Cervus elaphus*), and therefore are potential vectors for *T. cervi* in Europe. In the United States in 1974, Davies and Clark suggested that the vector for trypanosomes was the horse fly (*Chrysops* spp.) (Krinsky, 1975). More recently, the ked (*Lipoptena mazamae*) has been implicated as the vector for *T. cervi* (Samuel et al., 2001). *L. mazamae* is a common blood feeding insect found generally in the Southern United States and Central and South America. It has been found on WTD, antelope, and other exotic deer as well as cattle (Samuel et al., 2001).

Kingston et al. (1981) identified trypanosomes in a 1.8 kg fetus harvested from a trypanosome positive WTD dam, which suggested that a transplacental transmission of this parasite may be possible.

*T. cervi* has been documented by morphological examination of thin blood smears from numerous ungulate hosts in Europe and the United States. Within the United States, *T. cervi* has been recognized in elk (*Cervus canadensis)*, mule deer (*Odocoileus hemionus*), black-tailed deer (*O. h. columbianus*), WTD *(Odocoileus virginianus)*, moose (*Alces alces shirasi*), pronghorn (*Antilocapra americana*) and bison

(*Bison bison*) in Wyoming (Kingston et al., 1986; Kingston et al., 1981; Krinsky, 1975); WTD in Florida (Telford et al., 1991), North Carolina, Georgia, Arkansas, and Alabama (Davidson et al., 1983; Kingston et al., 1981); and in moose (*Alces alces gigas*) and reindeer (*Rangifer tarandus*) in Alaska (Kingston et al., 1985; Kingston et al., 1982).

*T. cervi* has been found to be ubiquitous in wild ungulates and for the most part has not been implicated as causing disease in these hosts. Levine et al. (1987) suggested that *T. cervi* may cause choroid plexitis in WTD, when they found high *T. cervi* parasitemia in afflicted animals. No definitive study was conducted, though.

*T. theileri* also has been considered to be nonpathogenic (Schlafer, 1979), although Cross (1972) and Cross et al. (1968) have implicated *T. theileri* in bovine leukocytosis and lymphocytosis. Carmichael (1926) observed *T. theileri* in cattle that had been serum inoculated for the manufacture of anti-rinderpest serum, although it is possible that trypanosme was injected along with the viral blood.

**Diagnosis**

Diagnosis is by visualization of parasite in blood as well as xenodiagnoses, where laboratory bred vectors feed on suspected animals and droppings are examined for presence of the parasite (Hoare, 1972). Giemsa stained blood smears are the most commonly used diagnostic tool for trypanosome visualization in the mammalian host. In order to diagnose the species of trypanosome found, blood smears are made and photomicrographs acquired and historically projected at a standard distance. The parasites are traced and measured with a calibrated map-wheel reader (Kingston et al., 1981). Alternatively, measurements from calibrated ocular micrometers under oil immersion may be used (Telford et al., 1991). Increasing, molecular methods are employed for species (and subspecies) differentiation (Bromidge et al., 1993; Garcia et al., 2011; Higo et al., 2007).

**Summary**

*T. cervi* has been found in a variety of ruminant species and in many countries, from Europe to the Americas. Generally, diagnosis is based on visualization of the parasite in blood smears. Other diagnostic methods available for *Trypanosoma* spp. include serologic analysis as well as molecular analysis. Serologic analyses include tests such as indirect fluorescent antibody test (IFA), which detects the presence of antibodies, but cannot detect with certainty active parasite infection (Gallatin et al., 2003). Genetic markers can be used to identify protozoal parasites and the small subunit ribosomal RNA (SSU rRNA) is currently one of the standard methods for genetic classification. The internal transcribed spacers (ITS) of ribosomal DNA (rDNA) have been found to be species specific and are able to distinguish parasites to the subspecies level (reviewed by Prichard and Tait, 2001). The ITS regions include highly conserved segments (Zahler et al., 1998). Collins and Allsopp (1999) found that the ITS genes of several *Theileria parva* isolates contained different combinations of sequence segments while the 5.8S regions were identical between species. *Trypanasoma* parasites obtained from different species of ungulates in the past have been found to be visually similar; but to date no genetic sequencing has been performed on these parasites in the United States. This study was conducted to look at different *Trypanosoma* spp. found in wild ungulates and compare 18S rRNA and ITS1, 5.8S, ITS2 rDNA sequences to those of *Trypanosoma* found in domestic cattle.

# CHAPTER II

# MOLECULAR IDENTIFICATION OF WTD DERIVED *BABESIA* SPECIES AND CULTURING BOVINE AND WTD DERIVED *BABESIA* SPECIES

## INTRODUCTION

There are a variety of *Babesia* species affecting different mammalian species. *Babesia bovis* is known to infect predominately cattle and is transmitted by the cattle fever ticks *Rhipicephalus (Boophilus) annulatus* and *Rhipicephalus (Boophilus) microplus*). The arthropod host injects the infective stage (sporozoites) of *B. bovis* into the mammalian host (Sauer et al., 1995). The sporozoite actively penetrates the mammalian erythrocyte. Once within the mammalian host cell, the sporozoite changes into a trophozoite, replicates, and forms haploid merozoites (Freeman et al., 2010).

Cattle fever ticks were eradicated from the United States in 1943 (Graham and Hourrigan, 1977; Howell et al., 2007). After eradication, a buffer zone of quarantined area, 800 km long and 0.4 to 16 km wide, was established in Texas along the Rio Grande from Brownsville to Del Rio (Graham and Hourrigan, 1977).

White-tailed deer (WTD) have been considered an alternate host for *B. bovis* (Cantu et al., 2007; Kuttler et al., 1972) and could possibly be infected without clinical signs (Cantu et al., 2007). Cattle fever ticks are able to complete their life cycle on WTD (Graham and Hourrigan, 1977; Pound et al., 2010) and successful infection of *B. bovis* in both WTD and cattle would make WTD a long-lasting reservoir for *B. bovis.* Wild WTD are able to travel between pastures and infect where cattle graze. Controlling the native free-ranging WTD population is very difficult and could make eradication of the disease problematic. Failure of tick eradication programs in Florida and Texas has been attributed by some researchers to WTD (Pound et al., 2010).

PCR examination has been developed to help identify the parasite in blood samples (Figueroa et al., 1993; Holman et al., 2011; Ramos et al., 2010). Culture has been used previously to study the parasite in vitro rather than in vivo, as well as increase the numbers of parasite in vitro (Holman et al., 1988; Levy and Ristic, 1980).

In the present study, WTD blood was analyzed for the presence of *B. bovis* 18S ribosomal DNA using PCR and thin film blood smear evaluation. Positive PCR products were sequenced and these sequences were compared to banked cattle *B. bovis* 18S rDNA sequences. Positive samples were cultured to determine the morphological features of any parasites found.

## MATERIALS AND METHODS

### WTD Blood Samples

WTD blood samples (N=161) were provided as a collaborative agreement with Dr. Adalberto Peréz de León (Director, USDA/ARS Knipling Bushland U.S. Livestock Insects Laboratory, Kerrville, TX), Dr. Greta Schuster (Texas AgriLife Research-Kingsville), and Dr. Susan Cooper (Texas AgriLife Research- Uvalde) from January to November 2010 (Ramos et al., 2011). WTD blood samples (N=52) were provided by Dr.

Adalberto Peréz de León from February to May 2011. All blood samples were collected by the collaborators for their studies under their appropriate animal use protocols. The WTD were of both sexes and were free-ranged or minimally managed. Blood was collected into ethylenediamine-tetraacetic acid (EDTA) blood tubes and shipped to Texas A&M University (College Station, TX) on ice. At Texas A&M University, the blood tubes were centrifuged at 800 X g for 15 min to separate red blood cells from plasma. A thin blood smear was made from the sedimented red blood cells in each EDTA tube and stained with Giemsa. These blood smears were evaluated under oil emersion at 1000x for the presence of parasites (Holman et al., 2010; Ramos et al., 2010).

**Cattle Blood Samples**

Cattle blood was obtained from ranched cattle of both sexes of various ages with differing proportions of Bonsmara stock (Bonsmara are a South African *Bos taurus* breed) residing in the southern Texas county of Starr, near the Mexican border. Blood was collected during normal round up and provided for this study as a collaborative project with Dr. Susan Cooper (Texas AgriLife Research-Uvalde). All animals were treated in accordance with the approved animal use protocol (PI Cooper, TAMU-Uvalde). Blood was collected into EDTA blood tubes and shipped to Texas A&M University (College Station, TX) on ice. At Texas A&M, the blood tubes were centrifuged at 800 X g for 15 min to separate red blood cells from plasma. A thin blood smear was made from the sedimented red blood cells in each EDTA tube and stained with Giemsa. These blood smears were evaluated under oil immersion at 1000x for the presence of parasites (Holman et al., 2010; Ramos et al., 2010).

**DNA Extraction**

From each of the EDTA blood tubes centrifuged as above, 0.2 ml packed RBC was removed and added to 1.8 ml Dulbecco's phosphate buffered saline (PBS) (Biowhittaker) and mixed in a 2 ml centrifuge tube. The sample tubes were then centrifuged at 1000 X g for 3 min. The supernatant was removed and discarded and DNA extracted from the pellet.

The DNA extractions were performed according to the FlexiGene DNA extraction protocol (Qiagen) with minor modifications. FG1 buffer (750 μl) was added to the washed red blood cells and the tubes were inverted several times. The samples were centrifuged at 15000 X g for 3 min. All supernatant was removed and discarded and FG2 buffer, containing proteinase, was added to the pellet. The samples were vortexed until all membranous strands were solubilized and then incubated in a 65 °C water bath for 5 min. An equal volume of isopropanol was added to the tube and the samples were mixed gently and centrifuged at 15000 X g for 3 min to pellet the DNA. The supernatant was removed and discarded and 150 μl 70% isopropanol was added to the pellet. After brief vortexing, the samples were centrifuged as above, the supernatant was removed and the DNA pellet was allowed to air dry. FG3 buffer

(30 μl) was added and the tubes placed in a 65 °C water bath for 10 min. Samples were incubated at room temperature overnight and stored at 4 °C until use.

The extracted DNA was reheated in a 65 °C water bath for 10 min and 2 μl used to determine quantity and quality of DNA present in each sample using spectrometry (NanoDropND-1000, Nanodrop Technologies, Wilmington, DE). Polymerase chain reaction (PCR) was performed on the extracted DNA (Ramos, 2010, Holman 2010).

**Polymerase Chain Reaction and Gel Electrophoresis for *Babesia bovis* 18S rRNA Gene**

One microliter (approximately 50-100 ng DNA) was mixed with 24 μl master mix containing primers A (forward, 5′-AACCTGGTTGATCCTGCCAG-3′) and B (reverse, 5′-GATCCTTCTGCAGGTTCACCTAC-3′), 10X PCR buffer, dNTP, $MgCl_2$, Taq polymerase, and sterile water in PCR tubes according to manufacturer's recommendations (Hi Fidelity Platinum Taq, Invitrogen). One microliter of a plasmid preparation containing the full-length *Babesia bigemina* 18S ribosomal RNA (cloned from *B. bigemina* from a previously determined positive cow) served as a positive control. No added DNA was used as a negative control.

Samples were placed in a Multigene (Labnet) thermocycler using 25 μl setting initially heated for 30 s at 94 °C and then 45 cycles of 94 °C for 15 s, 60 °C for 15 s, and then 68 °C for 2 min; the last cycle was 7 min at 68 °C and then held at 4 °C.

The sample amplicons plus the positive and negative controls were electrophoresed through a TBE buffered 1% agarose gel for about 20 min. The gel was rinsed with distilled water and immersed in ethidium bromide stain and allowed to incubate for 4 min with gentle agitation. The gel was once again rinsed and the image captured (FluorChem 8000, Alpha Innotech Corporation, San Leandro, CA).

All primary PCR products were diluted 1:10 or 1:20 with sterile water, depending on band intensity. The dilutions were used as template in a nested PCR. One microliter was added to 11.5 µl of a new master mix composed as above, but with primers BbovF600F and BbovR1500. One microliter of plasmid DNA containing an insert of *B. bovis* 18S rRNA gene (cloned from a *B. bovis* positive cow) was added to master mix and acted as a positive control. No added DNA was used as a negative control.

The reactions were placed in the Multigene thermocycler at 12 µl setting with initial denaturation 30 s at 94 °C and then 30 cycles of 94 °C for 15 s, 56 °C for 15 s, and then 68 °C for 1 min, the last cycle was 7 minutes at 68 °C and then the samples were held at 4 °C.

The nested PCR samples were electrophoresed through a TBE buffered 1% agarose gel. The gel was then rinsed and stained with ethidium bromide. The gel was then rinsed and imaged using gel documentation system (Holman et al., 2010; Ramos et al., 2010).

**Cloning and Sequencing**

Positive amplicons were cloned in TOPO PCR 2.1 and TOP 10 *Escherichia coli* following manufacturer's instructions (Invitrogen). Fresh PCR products (2 µl) were added to 0.5 µl salt solution and 0.5 µl TOPO vector. The mixture was incubated at room temperature for 10 min then added to One Shot *E. coli* thawed on ice. This was incubated on ice for 15 min and then heat shocked for 30 s at 42 °C. After heat shocking, 250 µl room temperature SOC media was added to the cells and the tubes were incubated at 37 °C at 200 rpm for 1 h. X-Gal (40 µl of 40 mg/ml solution) was applied to carbenicillin or kanamycin-containing (50 µg/ml) agar plates. The transformed cell suspension was divided and plated at different concentration on 2 plates and incubated overnight at 37 °C.

Color selected colonies were verified using colony PCR with m13F and m13R primers. Verified colonies were then expanded overnight in LB broth containing 50 µg/ml kanamycin or carbenicillin. Plasmid DNA was prepared from the transformed bacteria using plasmid mini-preps (Promega). The LB broth tubes were centrifuged to pellet the cells, the supernatant removed and discarded, and the pellet resuspended in 600 µl of TE or water and transferred to a 2 ml microcentrifuge tube. To the pellet, 100 µl of Cell Lysis Buffer was added and mixed, then, quickly, 350 µl of cold Neutralization Solution was added and mixed. The tubes were centrifuged at 15000 X g for 3 min. The supernatant was transferred to a mini-prep column in a collection tube and centrifuged for 30 s. The flow-through was discarded and 200 µl of Endotoxin

Removal Wash was added to the mini-column. The column was centrifuged for 30 s and 400 μl of Column Wash solution was added. The tube was centrifuged again and the mini-column was transferred to a new 2 ml tube. Elution Buffer (30 μl) was added to mini-column filter matrix and allowed to stand for 2.5 min, then centrifuged for 30 s. The column was transferred to a second 2 ml tube and 20 μl of Elution Buffer was added and allowed to stand before centrifugation. Plasmid concentrations were determined as described above and the plasmid inserts sequenced (Davis Sequencing, Davis, CA or Eton, San Diego, CA).

The plasmid sequence was trimmed from the obtained sequences, and the sequences were further trimmed to clearly readable bases, approximately 750-800 base stretches. The trimmed sequences were aligned and analyzed for contiguous sequences using Sequencher 4.2 software, then compared to banked sequences in the GenBank database using the NCBI BLAST tool (Altschul et al., 1990).

**Parasite Culture**

The blood samples positive for *B. bovis* were cultured in stationary phase microaerophilous system by placing 0.25 ml washed red blood cells from an original EDTA collection tube into one well of a 24- well culture plate containing 0.75 ml prepared culture medium. The culture medium consisted of 20% either bovine serum, fetal bovine serum (FBS), or WTD serum (provided by Dr. A. Blue McLendon, Texas A&M University), along with HL-1 medium (Biowhittaker), l-glutamine and antibiotic-antimycotic (200 μg/ml streptomycin, 200 U/ml penicillin, 50 μg/ml amphotericin B) solution (Holman et al., 1988; Holman et al., 1993). Each of the samples was plated in duplicate in culture wells. The plate was contained in a humidified microisolator containing a 5% carbon dioxide, 2% oxygen and 93% nitrogen atmosphere, and placed in an incubator held at 37 °C.

Culture media was changed daily; 0.8 ml of medium from above the erythrocyte layer was removed and 0.8 ml of fresh medium was added. The culture red blood cells were refreshed with 0.5 ml washed red blood cells from original EDTA tubes every 7 to 14 days depending on the appearance of the red blood cell layer in the culture. Microscopic evaluations of Giemsa-stained thin blood smears were performed daily (Holman et al., 1988, Holman et al., 1993).

**RESULTS**

On visual inspection of the blood smears under oil immersion (1000X), no *Babesia* piroplasms were identified. *Theileria* was identified on many of the smears in the forms of rings, piriforms, and oval forms.

Of the total 213 WTD samples screened for *B. bovis* by PCR, 8 showed bands at the proper base pair orientation for *B. bovis* on nested PCR (Table 1). This gave a 3.7% of possible positive *B. bovis* samples. On sequencing, there was a 99% similarity between the WTD derived *B. bovis*-like 18S rDNA regions to banked cattle *B. bovis* corresponding sequences (Ramos et al., 2010). Of the 103 cattle samples that were processed, none of them was positive for *B. bovis* by nested PCR (Table 2).

Four WTD samples screened February to May 2011 were positive and were cultured, as well as 4 previously identified positive samples (Ramos et al., 2010). Also, 2 samples with low packed cell volumes, suggestive of the presence of hemoparasites, were cultured. No definitive *Babesia* spp. was noted on daily smears. *Theileria* spp. in the form of rings, piriform and oval forms were noted in the WTD samples. No hemoparasites were noted in any of the cattle smears.

**Table 1.** *Babesia bovis* 18S WTD PCR results

| Date | Project Source | Animal ID | County | *B. bovis* |
|---|---|---|---|---|
| Oct-10 | APHIS | HF101 | Zapata | NEG |
| Oct-10 | APHIS | HF103 | Zapata | NEG |
| Oct-10 | APHIS | HF104 | Zapata | NEG |
| Oct-10 | APHIS | HF105 | Zapata | NEG |
| Oct-10 | APHIS | HF106 | Zapata | NEG |
| Oct-10 | APHIS | HF107 | Zapata | NEG |
| Oct-10 | APHIS | HF108 | Zapata | NEG |
| Oct-10 | APHIS | HF109 | Zapata | NEG |
| Oct-10 | APHIS | HF111 | Zapata | NEG |
| Oct-10 | APHIS | HF113 | Zapata | NEG |
| Oct-10 | APHIS | HF114 | Zapata | NEG |
| Oct-10 | APHIS | HF115 | Zapata | NEG |
| Oct-10 | APHIS | HF116 | Zapata | NEG |
| Oct-10 | APHIS | HF117 | Zapata | NEG |
| Oct-10 | APHIS | HF118 | Zapata | NEG |
| Oct-10 | APHIS | HF119 | Zapata | NEG |
| Oct-10 | APHIS | HF121 | Zapata | NEG |
| Oct-10 | APHIS | HF122 | Zapata | NEG |
| Oct-10 | APHIS | HF123 | Zapata | NEG |

**Table 1.** Continued

| Date | Project Source | Animal ID | County | *B. bovis* |
|---|---|---|---|---|
| Oct-10 | APHIS | HF126 | Zapata | NEG |
| Oct-10 | APHIS | HF127 | Zapata | NEG |
| Oct-10 | APHIS | HF128 | Zapata | NEG |
| Oct-10 | APHIS | HF129 | Zapata | NEG |
| Oct-10 | APHIS | HF130 | Zapata | NEG |
| Oct-10 | APHIS | HF131 | Zapata | NEG |
| Oct-10 | APHIS | HF132 | Zapata | NEG |
| Oct-10 | APHIS | HF133 | Zapata | NEG |
| Oct-10 | APHIS | HF134 | Zapata | NEG |
| Oct-10 | APHIS | HF135 | Zapata | NEG |
| Oct-10 | APHIS | HF136 | Zapata | NEG |
| Oct-10 | APHIS | HF137 | Zapata | NEG |
| Oct-10 | APHIS | HF138 | Zapata | NEG |
| Oct-10 | APHIS | HF139 | Zapata | NEG |
| Oct-10 | APHIS | HF140 | Zapata | NEG |
| Oct-10 | APHIS | HF141 | Zapata | NEG |
| Oct-10 | APHIS | HF142 | Zapata | NEG |
| Oct-10 | APHIS | HF143 | Zapata | NEG |
| Oct-10 | APHIS | HF144 | Zapata | NEG |
| Oct-10 | APHIS | HF145 | Zapata | NEG |
| Oct-10 | APHIS | HF146 | Zapata | NEG |
| Oct-10 | APHIS | HF147 | Zapata | NEG |
| Jul-10 | APHIS | HF1 | Zapata | NEG |
| Jul-10 | APHIS | HF2 | Zapata | NEG |
| Jul-10 | APHIS | HF3 | Zapata | NEG |
| Jul-10 | APHIS | HF4 | Zapata | NEG |
| Jul-10 | APHIS | HF5 | Zapata | NEG |
| Jul-10 | APHIS | HF6 | Zapata | NEG |
| Jul-10 | APHIS | HF7 | Zapata | NEG |
| Jul-10 | APHIS | HF8 | Zapata | NEG |
| Jul-10 | APHIS | HF9 | Zapata | NEG |
| Jul-10 | APHIS | HF10 | Zapata | NEG |
| Jul-10 | APHIS | HF11 | Zapata | NEG |
| Jul-10 | APHIS | HF12 | Zapata | NEG |
| Jul-10 | APHIS | HF13 | Zapata | NEG |
| Jul-10 | APHIS | HF14 | Zapata | NEG |
| Jul-10 | APHIS | HF15 | Zapata | NEG |
| Jul-10 | APHIS | HF16 | Zapata | NEG |

**Table 1.** Continued

| Date | Project Source | Animal ID | County | *B. bovis* |
|---|---|---|---|---|
| Jul-10 | APHIS | HF17 | Zapata | NEG |
| Jul-10 | APHIS | HF18 | Zapata | NEG |
| Jul-10 | APHIS | HF19 | Zapata | NEG |
| Jul-10 | APHIS | HF20 | Zapata | NEG |
| Jul-10 | APHIS | HF21 | Zapata | POS |
| Jul-10 | APHIS | HF22 | Zapata | NEG |
| Jul-10 | APHIS | HF23 | Zapata | NEG |
| Jul-10 | APHIS | HF24 | Zapata | NEG |
| Jul-10 | APHIS | HF25 | Zapata | NEG |
| Jul-10 | APHIS | HF26 | Zapata | NEG |
| Jul-10 | APHIS | HF27 | Zapata | NEG |
| Jul-10 | APHIS | HF28 | Zapata | NEG |
| Jul-10 | APHIS | HF29 | Zapata | NEG |
| Jul-10 | APHIS | HF30 | Zapata | NEG |
| Oct-10 | APHIS | SL61 | Zapata | NEG |
| Oct-10 | APHIS | SL62 | Zapata | NEG |
| Oct-10 | APHIS | SL63 | Zapata | NEG |
| Oct-10 | APHIS | SL64 | Zapata | NEG |
| Oct-10 | APHIS | SL65 | Zapata | NEG |
| Oct-10 | APHIS | SL66 | Zapata | NEG |
| Oct-10 | APHIS | SL67 | Zapata | NEG |
| Oct-10 | APHIS | SL68 | Zapata | NEG |
| Oct-10 | APHIS | SL69 | Zapata | NEG |
| Oct-10 | APHIS | SL71 | Zapata | NEG |
| Oct-10 | APHIS | SL72 | Zapata | NEG |
| Oct-10 | APHIS | SL73 | Zapata | NEG |
| Oct-10 | APHIS | SL74 | Zapata | NEG |
| Oct-10 | APHIS | SL75 | Zapata | NEG |
| Oct-10 | APHIS | SL76 | Zapata | NEG |
| Oct-10 | APHIS | SL77 | Zapata | NEG |
| Oct-10 | APHIS | SL78 | Zapata | NEG |
| Oct-10 | APHIS | SL79 | Zapata | NEG |
| Oct-10 | APHIS | SL80 | Zapata | NEG |
| Jul-10 | APHIS | NL1 | Zapata | NEG |
| Jul-10 | APHIS | NL2 | Zapata | NEG |
| Jul-10 | APHIS | NL3 | Zapata | NEG |
| Jul-10 | APHIS | NL4 | Zapata | NEG |
| Jul-10 | APHIS | NL5 | Zapata | NEG |

**Table 1.** Continued

| Date | Project Source | Animal ID | County | *B. bovis* |
|---|---|---|---|---|
| Jul-10 | APHIS | NL6 | Zapata | NEG |
| Jul-10 | APHIS | NL7 | Zapata | NEG |
| Jul-10 | APHIS | NL8 | Zapata | NEG |
| Jul-10 | APHIS | NL9 | Zapata | NEG |
| Jul-10 | APHIS | NL10 | Zapata | NEG |
| Jul-10 | APHIS | NL11 | Zapata | NEG |
| Jul-10 | APHIS | NL12 | Zapata | NEG |
| Jul-10 | APHIS | NL13 | Zapata | NEG |
| Jul-10 | APHIS | NL14 | Zapata | NEG |
| Jul-10 | APHIS | NL15 | Zapata | NEG |
| Jul-10 | APHIS | NL81 | Zapata | NEG |
| Jul-10 | APHIS | NL82 | Zapata | NEG |
| Jul-10 | APHIS | NL83 | Zapata | NEG |
| Jul-10 | APHIS | NL84 | Zapata | NEG |
| Jul-10 | APHIS | NL85 | Zapata | NEG |
| Jul-10 | APHIS | NL86 | Zapata | NEG |
| Jul-10 | APHIS | NL87 | Zapata | NEG |
| Jul-10 | APHIS | NL88 | Zapata | NEG |
| Jul-10 | APHIS | NL89 | Zapata | NEG |
| Jul-10 | APHIS | NL90 | Zapata | NEG |
| Jul-10 | APHIS | NL91 | Zapata | NEG |
| Jul-10 | APHIS | NL92 | Zapata | NEG |
| Jul-10 | APHIS | NL94 | Zapata | NEG |
| Jul-10 | APHIS | NL95 | Zapata | NEG |
| Jul-10 | APHIS | NL96 | Zapata | NEG |
| Jul-10 | APHIS | NL97 | Zapata | NEG |
| Jul-10 | APHIS | NL98 | Zapata | NEG |
| Jul-10 | APHIS | NL99 | Zapata | NEG |
| Jan-10 | TX AgriLife | W2 | Webb | NEG |
| Jan-10 | TX AgriLife | W3 | Webb | NEG |
| Jan-10 | TX AgriLife | W4 | Webb | NEG |
| Jan-10 | TX AgriLife | W5 | Webb | NEG |
| Jan-10 | TX AgriLife | W6 | Webb | NEG |
| Jan-10 | TX AgriLife | W7 | Webb | NEG |
| Jan-10 | TX AgriLife | W8 | Webb | NEG |
| Jan-10 | TX AgriLife | W9 | Webb | NEG |
| Jan-10 | TX AgriLife | W11 | Webb | NEG |

**Table 1.** Continued

| Date | Project Source | Animal ID | County | *B. bovis* |
|---|---|---|---|---|
| Jan-10 | TX AgriLife | W12 | Webb | NEG |
| Jan-10 | TX AgriLife | W13 | Webb | NEG |
| Jan-10 | TX AgriLife | W14 | Webb | NEG |
| Jan-10 | TX AgriLife | W15 | Webb | NEG |
| Jan-10 | TX AgriLife | W16 | Webb | NEG |
| Jan-10 | TX AgriLife | W17 | Webb | NEG |
| Jan-10 | TX AgriLife | W18 | Webb | NEG |
| Jan-10 | TX AgriLife | W19 | Webb | NEG |
| Jan-10 | TX AgriLife | W20 | Webb | NEG |
| Jan-10 | TX AgriLife | W21 | Webb | NEG |
| Jan-10 | TX AgriLife | W22 | Webb | NEG |
| Jan-10 | TX AgriLife | W23 | Webb | POS |
| Jan-10 | TX AgriLife | W24 | Webb | NEG |
| Jan-10 | TX AgriLife | W25 | Webb | POS |
| Jan-10 | TX AgriLife | W26 | Webb | NEG |
| Jul-10 | APHIS | SL2 | Zapata | NEG |
| Jul-10 | APHIS | SL3 | Zapata | NEG |
| Jul-10 | APHIS | SL4 | Zapata | NEG |
| Jul-10 | APHIS | SL5 | Zapata | NEG |
| Jul-10 | APHIS | SL6 | Zapata | NEG |
| Jul-10 | APHIS | SL7 | Zapata | NEG |
| Jul-10 | APHIS | SL8 | Zapata | NEG |
| Jul-10 | APHIS | SL9 | Zapata | NEG |
| Jul-10 | APHIS | SL10 | Zapata | NEG |
| Jul-10 | APHIS | SL11 | Zapata | POS |
| Jul-10 | APHIS | SL12 | Zapata | NEG |
| Jul-10 | APHIS | SL13 | Zapata | NEG |
| Jul-10 | APHIS | SL14 | Zapata | NEG |
| Jul-10 | APHIS | SL15 | Zapata | NEG |
| May-11 | USDA | A1 | Zapata | NEG |
| May-11 | USDA | A2 | Zapata | NEG |
| May-11 | USDA | A3 | Zapata | POS |
| May-11 | USDA | A4 | Zapata | NEG |
| May-11 | USDA | A5 | Zapata | NEG |
| May-11 | USDA | A6 | Zapata | NEG |
| May-11 | USDA | A7 | Zapata | NEG |
| May-11 | USDA | A8 | Zapata | NEG |

**Table 1.** Continued

| Date | Project Source | Animal ID | County | *B. bovis* |
|---|---|---|---|---|
| May-11 | USDA | A9 | Zapata | NEG |
| May-11 | USDA | A10 | Zapata | NEG |
| May-11 | USDA | A11 | Zapata | NEG |
| May-11 | USDA | A12 | Zapata | NEG |
| May-11 | USDA | A13 | Zapata | NEG |
| May-11 | USDA | A14 | Zapata | NEG |
| May-11 | USDA | A15 | Zapata | NEG |
| May-11 | USDA | A16 | Zapata | NEG |
| May-11 | USDA | A17 | Zapata | NEG |
| May-11 | USDA | A18 | Zapata | NEG |
| May-11 | USDA | A19 | Zapata | NEG |
| May-11 | USDA | A20 | Zapata | NEG |
| May-11 | USDA | A21 | Zapata | POS |
| May-11 | USDA | A22 | Zapata | POS |
| May-11 | USDA | A23 | Zapata | NEG |
| May-11 | USDA | A24 | Zapata | NEG |
| May-11 | USDA | A25 | Zapata | NEG |
| May-11 | USDA | A26 | Zapata | NEG |
| May-11 | USDA | A27 | Zapata | NEG |
| May-11 | USDA | A28 | Zapata | NEG |
| May-11 | USDA | A29 | Zapata | NEG |
| May-11 | USDA | A30 | Zapata | NEG |
| May-11 | USDA | A31 | Zapata | NEG |
| May-11 | USDA | A32 | Zapata | NEG |
| May-11 | USDA | A33 | Zapata | POS |
| May-11 | USDA | A34 | Zapata | NEG |
| May-11 | USDA | A35 | Zapata | NEG |
| Feb-11 | USDA | 147 | Zapata | NEG |
| Feb-11 | USDA | 148 | Zapata | NEG |
| Feb-11 | USDA | 149 | Zapata | NEG |
| Feb-11 | USDA | 150 | Zapata | NEG |
| Feb-11 | USDA | 151 | Zapata | NEG |
| Feb-11 | USDA | 152 | Zapata | NEG |
| Feb-11 | USDA | 153 | Zapata | NEG |
| Feb-11 | USDA | 154 | Zapata | NEG |
| Feb-11 | USDA | 155 | Zapata | NEG |
| Feb-11 | USDA | 156 | Zapata | NEG |

**Table 1.** Continued

| Date | Project Source | Animal ID | County | *B. bovis* |
|---|---|---|---|---|
| Feb-11 | USDA | 157 | Zapata | NEG |
| Feb-11 | USDA | 158 | Zapata | NEG |
| Feb-11 | USDA | 159 | Zapata | NEG |
| Feb-11 | USDA | 160 | Zapata | NEG |
| Feb-11 | USDA | 161 | Zapata | NEG |
| Feb-11 | USDA | 162 | Zapata | NEG |
| Feb-11 | USDA | 163 | Zapata | NEG |

**Table 2.** *Babesia bovis* 18S cattle PCR results

| Date (Month/Year) | Species | Source | Animal ID | County | *B. bovis* |
|---|---|---|---|---|---|
| Mar-10 | Cattle | USDA | 4-2 | Starr | NEG |
| Mar-10 | Cattle | USDA | 2 | Starr | NEG |
| Mar-10 | Cattle | USDA | 4 | Starr | NEG |
| Mar-10 | Cattle | USDA | 9 | Starr | NEG |
| Mar-10 | Cattle | USDA | 12 | Starr | NEG |
| Mar-10 | Cattle | USDA | 15 | Starr | NEG |
| Mar-10 | Cattle | USDA | 16 | Starr | NEG |
| Mar-10 | Cattle | USDA | 24 | Starr | NEG |
| Mar-10 | Cattle | USDA | 30 | Starr | NEG |
| Mar-10 | Cattle | USDA | 31 | Starr | NEG |
| Mar-10 | Cattle | USDA | 32 | Starr | NEG |
| Mar-10 | Cattle | USDA | 34 | Starr | NEG |
| Mar-10 | Cattle | USDA | 36 | Starr | NEG |
| Mar-10 | Cattle | USDA | 45 | Starr | NEG |
| Mar-10 | Cattle | USDA | 46 | Starr | NEG |
| Mar-10 | Cattle | USDA | 47 | Starr | NEG |
| Mar-10 | Cattle | USDA | 48 | Starr | NEG |
| Mar-10 | Cattle | USDA | 56 | Starr | NEG |
| Mar-10 | Cattle | USDA | 60 | Starr | NEG |
| Mar-10 | Cattle | USDA | 63 | Starr | NEG |
| Mar-10 | Cattle | USDA | 66 | Starr | NEG |
| Mar-10 | Cattle | USDA | 67 | Starr | NEG |
| Mar-10 | Cattle | USDA | 73 | Starr | NEG |
| Mar-10 | Cattle | USDA | 95 | Starr | NEG |
| Mar-10 | Cattle | USDA | 96 | Starr | NEG |
| Mar-10 | Cattle | USDA | 104 | Starr | NEG |
| Mar-10 | Cattle | USDA | 107 | Starr | NEG |
| Mar-10 | Cattle | USDA | 108 | Starr | NEG |
| Mar-10 | Cattle | USDA | 110 | Starr | NEG |
| Mar-10 | Cattle | USDA | 117 | Starr | NEG |
| Mar-10 | Cattle | USDA | 124 | Starr | NEG |
| Mar-10 | Cattle | USDA | 125 | Starr | NEG |
| Mar-10 | Cattle | USDA | 127 | Starr | NEG |
| Mar-10 | Cattle | USDA | 128 | Starr | NEG |
| Mar-10 | Cattle | USDA | 130 | Starr | NEG |
| Mar-10 | Cattle | USDA | 131 | Starr | NEG |
| Mar-10 | Cattle | USDA | 133 | Starr | NEG |

**Table 2.** Continued

| Date (Month/Year) | Species | Source | Animal ID | County | *B. bovis* |
|---|---|---|---|---|---|
| Mar-10 | Cattle | USDA | 136 | Starr | NEG |
| Mar-10 | Cattle | USDA | 139 | Starr | NEG |
| Mar-10 | Cattle | USDA | 158 | Starr | NEG |
| Mar-10 | Cattle | USDA | 165 | Starr | NEG |
| Mar-10 | Cattle | USDA | 169 | Starr | NEG |
| Mar-10 | Cattle | USDA | 179 | Starr | NEG |
| Mar-10 | Cattle | USDA | 196 | Starr | NEG |
| Mar-10 | Cattle | USDA | 208 | Starr | NEG |
| Mar-10 | Cattle | USDA | 219 | Starr | NEG |
| Mar-10 | Cattle | USDA | 1123 | Starr | NEG |
| Mar-10 | Cattle | USDA | 2009 | Starr | NEG |
| Mar-10 | Cattle | USDA | 2031 | Starr | NEG |
| Mar-10 | Cattle | USDA | 2048 | Starr | NEG |
| Mar-10 | Cattle | USDA | 2049 | Starr | NEG |
| Mar-10 | Cattle | USDA | 2060 | Starr | NEG |
| Mar-10 | Cattle | USDA | 2063 | Starr | NEG |
| Mar-10 | Cattle | USDA | 2068 | Starr | NEG |
| Mar-10 | Cattle | USDA | 2070 | Starr | NEG |
| Mar-10 | Cattle | USDA | 2073 | Starr | NEG |
| Mar-10 | Cattle | USDA | 2082 | Starr | NEG |
| Mar-10 | Cattle | USDA | 2085 | Starr | NEG |
| Mar-10 | Cattle | USDA | 2092 | Starr | NEG |
| Mar-10 | Cattle | USDA | 2095 | Starr | NEG |
| Mar-10 | Cattle | USDA | 2096 | Starr | NEG |
| Mar-10 | Cattle | USDA | 2101 | Starr | NEG |
| Mar-10 | Cattle | USDA | 2110 | Starr | NEG |
| Mar-10 | Cattle | USDA | 2112 | Starr | NEG |
| Mar-10 | Cattle | USDA | 2124 | Starr | NEG |
| Mar-10 | Cattle | USDA | 2125 | Starr | NEG |
| Mar-10 | Cattle | USDA | 2126 | Starr | NEG |
| Mar-10 | Cattle | USDA | 2127 | Starr | NEG |
| Mar-10 | Cattle | USDA | 2138 | Starr | NEG |
| Mar-10 | Cattle | USDA | 2141 | Starr | NEG |
| Mar-10 | Cattle | USDA | 2142 | Starr | NEG |
| Mar-10 | Cattle | USDA | 2166 | Starr | NEG |
| Mar-10 | Cattle | USDA | 2170 | Starr | NEG |
| Mar-10 | Cattle | USDA | 2176 | Starr | NEG |

**Table 2.** Continued

| Date (Month/Year) | Species | Source | Animal ID | County | *B. bovis* |
|---|---|---|---|---|---|
| Mar-10 | Cattle | USDA | 2185 | Starr | NEG |
| Mar-10 | Cattle | USDA | 2186 | Starr | NEG |
| Mar-10 | Cattle | USDA | 2199 | Starr | NEG |
| Mar-10 | Cattle | USDA | 2201 | Starr | NEG |
| Mar-10 | Cattle | USDA | 3005 | Starr | NEG |
| Mar-10 | Cattle | USDA | 3006 | Starr | NEG |
| Mar-10 | Cattle | USDA | 3023 | Starr | NEG |
| Mar-10 | Cattle | USDA | 3024 | Starr | NEG |
| Mar-10 | Cattle | USDA | 3026 | Starr | NEG |
| Mar-10 | Cattle | USDA | 3040 | Starr | NEG |
| Mar-10 | Cattle | USDA | 3071 | Starr | NEG |
| Mar-10 | Cattle | USDA | 3074 | Starr | NEG |
| Mar-10 | Cattle | USDA | 3099 | Starr | NEG |
| Mar-10 | Cattle | USDA | 3135 | Starr | NEG |
| Mar-10 | Cattle | USDA | 3504 | Starr | NEG |
| Mar-10 | Cattle | USDA | 3520 | Starr | NEG |
| Mar-10 | Cattle | USDA | 3535 | Starr | NEG |
| Mar-10 | Cattle | USDA | 3539 | Starr | NEG |
| Mar-10 | Cattle | USDA | 3571 | Starr | NEG |
| Mar-10 | Cattle | USDA | 3577 | Starr | NEG |
| Mar-10 | Cattle | USDA | 3635 | Starr | NEG |
| Mar-10 | Cattle | USDA | 3641 | Starr | NEG |
| Mar-10 | Cattle | USDA | 4253 | Starr | NEG |
| Mar-10 | Cattle | USDA | 9025 | Starr | NEG |
| Mar-10 | Cattle | USDA | 9082 | Starr | NEG |
| Mar-10 | Cattle | USDA | 9215 | Starr | NEG |
| Mar-10 | Cattle | USDA | 9311 | Starr | NEG |

## DISCUSSION

It is still unclear whether WTD could be reservoirs for cattle *B. bovis*. Molecular and serologic evidence have suggested that WTD may carry the causative agent of bovine babesiosis (Cantu et al., 2007; Ramos et al., 2010; Holman et al., 2010). Amplification of the *B. bovis* small subunit ribosomal RNA gene identified WTD in two southern counties of Texas as possible carriers of this parasite in the current study. However, sequence analysis of the gene was unable to unequivocally demonstrate that the parasite WTD carried was *B. bovis.* The WTD samples cultured in this study unfortunately did not produce a detectable *Babesia* parasitemia in in vitro culture so that the morphology of the WTD parasite is unknown. No PCR positives were found in the cattle samples from the same county, which would have provided a *B. bovis* 18S rRNA gene comparative sequence data.

Cross-reactivity of a closely related *Babesia* species is a possibility in WTD (Holman et al., 2010). There is potential that the PCR reaction is sensitive to a *Babesia* parasite that is very closely related to cattle *B. bovis* and this related parasite has only a 1% difference in the 18S rRNA region.

This study looked at WTD and cattle from more southern areas in Texas (Fig. 1), as well as higher numbers of animals than in previously published studies (Ramos et al., 2010; Holman et al., 2010). All of the counties where cattle and WTD samples were obtained for this study are in the quarantine buffer zone of the Mexican border and have had cattle fever tick outbreaks. Webb and Zapata counties both have had *Rhipicephalus (Boophilus) annulatus* outbreaks between 1994 and 2004, while Webb, Zapata, and Starr counties all have had *Rhipicephalus (Boophilus) microplus* outbreaks (Estrada-Pena and Venzal, 2006). WTD are able to harbor cattle fever ticks and the ticks are able to complete their life cycle on the WTD (Graham and Hourrigan, 1977; Pound et al., 2010). It is still conceivable that while cattle fever ticks are parasitizing WTD, they are also passing *B. bovis*. Whether or not *B. bovis* is able to complete its lifecycle in the WTD is still unknown.

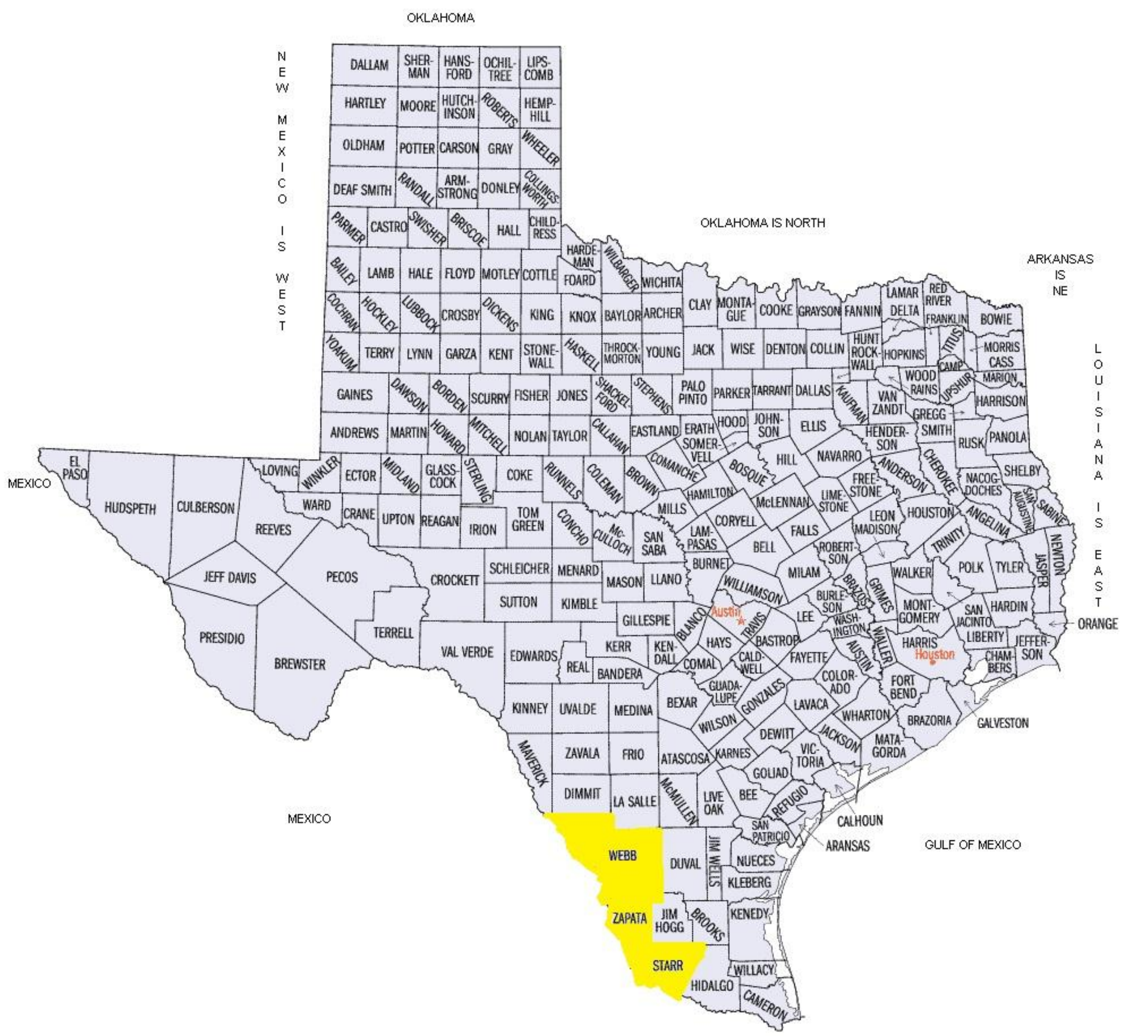


**Figure 1.** Map of Texas highlighting Webb, Starr, and Zapata counties

In the culture, it is conceivable that *B. bovis* growth did occur from the WTD blood samples, but the daily smears did not show positive *B. bovis* forms. This may have been due to the parasites congregating in a certain area of the well that was not sampled for microscopic evaluation. It could also be that the ring, pyriform, and oval forms seen were mistaken for *Theileria* spp. The only way to definitively differentiate *B. bovis* from *Theileria* microscopically is to find paired pyriforms (haploid merozoites), none of which were seen. It is also possible that during the growth phase, the *Theileria* spp. were better able to grow and outperformed the *B. bovis*, causing the *B. bovis* to be overwhelmed and unable to grow alongside the *Theileria*.

Since PCR is a sensitive test capable of detecting very low parasitemias, it is possible that the growth of *Babesia* in culture failed due to low parasitemia in the original samples. Even with a low parasitemia, culturing of the samples would be a useful way to expand the population. Holman et al (1998) were able to culture *Babesia equi* from samples where the parasitemia could not be detected on the original blood smear or by indirect fluorescent antibody test. However, it is a possibility that the PCR test only detected the genetic material from dead or degenerating parasites, which when these parasites were placed into culture, were unable to grow and reproduce. Although performed in a timely manner, it may be that the time lag between blood collection, DNA extraction, PCR and actually initiating the cultures was too long to maintain the viability of the parasites. In future culturing attempts, it may be necessary to determine *B. bovis* positive WTD by PCR and then collect fresh blood samples from the positive WTD for immediate culture.

Another possibility for the failure of the WTD *B. bovis* parasites to grow in culture could be due to the parasites' adaptation to WTD. It is feasible that the *B. bovis* species have acclimatized to the WTD and are unable to grow in culture conditions developed specifically for cattle derived *B. bovis*. Holman et al. (2005) found that culture conditions optimized for several *Babesia* spp. were not able to support the growth of a *Babesia* spp. found in the Eastern Cottontail rabbit (*Sylvilagus floridanus*). On the other hand, if the *B. bovis* PCR positives were not actually *B. bovis*, the culture growth conditions cultivated for true *B. bovis* may not allow growth of another parasite species. In order to cultivate the *B. bovis* PCR positives originating from WTD, it may be necessary to investigate different culture options. In this study, the red blood cells were refreshed every 7 to 14 days, which may have not been often enough. Also, the amount of serum to HL-1 medium supplemented with antibiotics-antimycotics and l-glutamine may not have been optimal for these parasites. In order to be able to grow these parasites it may be necessary to organize trials with different culture conditions to determine the optimum environment for growth of the parasite.

For future studies, more positive WTD samples need to be obtained and sequenced. It may be helpful to sequence and analyze other regions of the ribosomal genes, such as the ITS1, 5.8S, and ITS2 regions. These regions can be more divergent between species which could give more information into how similar the WTD obtained parasite is to cattle *B. bovis*. Positive samples will also need to be successfully cultured in order to visualize and compare this WTD *Babesia* species with the *B. bovis* obtained from cattle. In vivo studies using PCR positive *B. bovis* isolates obtained from WTD and cattle need to be inoculated into cross species. Once *B. bovis* like species can be cultured from WTD, one of the next phases would be to determine if these parasites can complete their life cycle in cattle. Another phase would include inoculating *B. bovis* originating from cattle into WTD to determine whether WTD can perpetuate the parasite or if they are a dead end host.

# CHAPTER III

# SEQUENCING RIBOSOMAL DNA FROM *TRYPANOSOMA* SPP. OBTAINED FROM WTD AND ELK IN COMPARISON TO *TRYPANOSOMA* SPP. OBTAINED FROM CATTLE

## INTRODUCTION

Before 1975, trypanosomes of white-tailed deer were classified as *Trypanosoma theileri*. In 1975, Kingston and Morton classified trypomastigotes from elk (*Cervus canadensis)* and mule deer (*Odocoileus hemionus*) as *Trypanosoma cervi* based on morphological and biological distinctions (Matthews et al., 1977). *T. theileri* occurs most commonly in the blood of cattle (Levine, 1985) and generally does not cause clinical signs, unless the animal is immunocompromised by another disease (Carmichael, 1926; Cross, 1972; Cross et al., 1968). *T. cervi* has been found to be ubiquitous in wild ungulates in the United States, including elk (*Cervus canadensis)*, mule deer (*Odocoileus hemionus*), black-tailed deer (*Odocoileus hemionus columbianus*), white-tailed deer *(Odocoileus virginianus)*, moose (*Alces alces shirasi*), pronghorn (*Antilocapra americana*) and bison (*Bison bison*) (Kingston et al., 1986; Kingston et al., 1981; Krinsky, 1975). For the most part, this parasite has not been implicated as causing disease in wild ungulates. Previous researchers have attempted to inoculate *T. cervi* positive blood obtained from elk into naïve splenectomized calves (Kingston and Morton, 1975a; Matthews et al., 1977). This did not lead to detectable infection in the naïve animals.

In the U.S. *Trypanosomes* in the past have only been diagnosed by visualization methods rather than genetic methods, thus it could be that while morphologically similar, the parasites identified as *T. cervi* are genetically different between species. Comparing the ribosomal DNA genetic sequence from WTD *Trypanosoma* to *Trypanosoma* rDNA sequences obtained from other hosts would give provide evidence of whether these parasites are conspecific or separate species.

In this study, *Trypanosoma* sequences obtained from WTD, elk, and cattle isolates were compared using 18S ribosomal DNA (rDNA) and the genomic region spanning the ribosomal RNA intervening transcribed spacer-1, the 5.8S ribosomal RNA gene, and intervening transcribed spacer-2 (ITS1-5.8S-ITS2).

## MATERIALS AND METHODS

### DNA samples

DNA extracted from WTD and cattle blood was provided from previous projects (Chapter II). The extracted DNA was heated in a 65 °C water bath for 10 min and 2 µl used to determine quantity and quality of DNA present in each sample using spectrometry (NanoDropND-1000, Nanodrop Technologies, Wilmington, DE).

**Amplification of the *Trypanosoma* 18S rRNA Gene**

Polymerase chain reaction (PCR) was performed on the extracted DNA (Ramos, 2010, Holman 2010). One microliter DNA (approximately 50-100 ng) was mixed with 24 µl master mix containing primers TrypF (5'-GTCATATGCTTGTTTCAAGGACT-TAG-3') and primer TrypR (5' –GACTTTTGCTTCCTCTATTGAAGAAAT- 3') (Table 3), 10X PCR buffer, dNTP, $MgCl_2$, Taq polymerase, and sterile water in PCR tubes according to manufacturer's instructions (Hi Fidelity Platinum Taq, Invitrogen). The specific primers were designed from *Trypanosoma theileri* 18S rRNA gene sequence GenBank Accession No. AJ009164, and checked for specificity and conservation in related *Trypanosome* sequences in the GenBank database. One microliter of 18S rRNA gene amplicon from a previous positive WTD *Trypanosoma* sp. PCR was added to master mix and served as a positive control. No added DNA was used as a negative control.

Samples were placed in a Multigene thermocyler (Labnet International, Inc., Edison, NJ) using the 25 µl setting and initially heated for 30 s at 94 °C followed by 45 cycles of 94 °C for 15 s, 55 °C for 15 s, and then 68 °C for 2 min; the last cycle was 7 min at 68 °C and then held at 4 °C.

The WTD and cattle amplification products plus the positive and negative controls were electrophoresed through a 1% TBE buffered agarose gel for about 20 min. The gel was rinsed with distilled water and immersed in ethidium bromide stain and allowed to incubate for 4 min with gentle agitation. The gel was once again rinsed and the image captured (FluorChem 8000, Alpha Innotech Corporation, San Leandro, CA).

**Table 3.** Primers

| *Name* | *Sequence 5′-3′* | *Target* | *°C Annealing Temperature* |
|---|---|---|---|
| | | *18S rRNA Gene* | |
| TrypF | GTCATATGCTTGTTTCAAGGACTTAG | 5' end | 55 |
| TrypR | GACTTTTGCTTCCTCTATTGAAGAAAT | 3' end | 55 |
| TrypF150 | GCCAAGCTAATACATGAACCAAAGGG | 5' nested | 57 |
| TrypR1500 | ATGCACGTAAATTGATATCCATTGGC | 3' nested | 57 |
| | | *ITS1-5.8S-ITS2* | |
| ITSFTryp | CACCCGCGGTAATTCCAGC | 18S position | 55 |
| ITSRTryp | GCGGGTAGTCCTGCCAAACACTCAGGTCTG | 28S position | 55 |
| TrypITSFN | CACCCGCGGTAATTCCAGC | 18S position | 57 |
| TrypITSRN | TGACAAAGATTGCAGATACGCGT | 28S position | 57 |

For confirmation of amplification of *Trypanosoma* 18S rRNA genes in the primary PCR, amplicons were diluted 1:10 or 1:20 with sterile water, depending on band intensity. The dilutions were used as template in nested PCR. One microliter was placed in 11.5 μl new master mix composed as above, but containing primers TrypF150 (5' –GCCAAGCTAATACATGAACCAAAGGG- 3') and TrypR1500 (5' –ATGCACG TAAATTGATATCCATTGGC- 3') (Table 3) (designed as described for the outer primers, above). The controls were as described above.

The reactions were placed in the thermocycler at the 12 µl setting starting for 30 s at 94 °C and then 30 cycles of 94 °C for 15 s, 57 °C for 15 s, and then 68 °C for 1.5 min; the last cycle was 7 min at 68 °C and then held at 4 °C. The nested PCR products were electrophoresed through a 1% buffered agarose gel. The gel was then rinsed and stained with ethidium bromide. The gel was then rinsed and imaged using gel documentation system.

**Amplification of the *Trypanosoma* rRNA Genomic Region Spanning the Intervening Transcribed Spacer 1, 5.8S rRNA Gene, and Intervening Transcribed Spacer 2 (ITS1-5.8S-ITS2)**

One microliter DNA (approximately 50-100 ng) was mixed with 24 µl master mix containing primers ITSF (5' – CACCCGCGGTAATTCCAGC – 3') and ITSR (5' - GCGGGTAGTCCTGCCAAACACTCAGGTCTG – 3') (designed from GenBank Accession nos. AB569249 and AY773700 as described above) (Table 3), 10X PCR buffer, dNTP, $MgCl_2$, Taq polymerase, and sterile water in PCR tubes according to manufacturer's instructions (Hi Fidelity Platinum Taq, Invitrogen). No added DNA was used as a negative control.

Samples were placed in a Multigene thermocycler using the 25 µl setting and initially heated for 30 s at 94 °C and then 45 cycles of 94 °C for 15 s, 55 °C for 15 s, and then 68 °C for 2 min; the last cycle was 7 min at 68 °C and then held at 4 °C. Resulting products were evaluated by agarose gel electrophoresis as described above.

For confirmation of amplification of *Trypanosoma* 18S rRNA genes in the primary PCR, amplicons were diluted 1:10 or 1:20 with sterile water, depending on band intensity. The dilutions were used as template in nested PCR. One microliter was placed in 11.5 μl new master mix composed as above, but containing primers TrypITS18SFN (5' –CACCCGCGGTAATTCCAGC- 3') and TrypITSRN (5'-TGACAAA-GATTGCAGATACGCGT- 3') (designed as described above for the outer primers) (Table 3). One microliter *Trypanosoma* spp. ITS rDNA amplicon was added to master mix and acted as a positive control. No added DNA was used as the negative control.

The reactions were placed in the Multigene thermocycler at 12 μl setting and heated for 30 s at 94 °C followed by 30 cycles of 94 °C for 15 s, 57 °C for 15 s, and then 68 °C for 1.5 minutes; the last cycle was 7 min at 68 °C and then held at 4 °C.

The nested PCR products were electrophoresed through a 1% buffered agarose gel. The gel was then rinsed and stained with ethidium bromide. The gel was then rinsed and imaged as previously described.

**Cloning and Sequencing**

The 18S rDNA and ITS1-5.8S-ITS2 region positive amplicons were cloned into TOPO PCR 2.1 and TOP 10 *Escherichia coli* transformed following manufacturer's instructions as described previously (Chapter II).

Color selected colonies were verified using colony PCR with m13F and m13R primers. Verified colonies were then expanded overnight in LB broth containing 50 μg/ml ampicillin or kanamycin. Plasmid DNA was prepared from the transformed bacteria using plasmid mini-preps (Promega or Bio Basic Inc). The overnight cultures were centrifuged to pellet the cells, the supernatant removed and discarded. The Promega plasmid mini-preps procedures were described in Chapter II. For the Bio Basic EZ-10 spin BS614 minipreps, the pellet was resuspended in 100 μl of Solution I and transferred to 2 ml tube and allowed to set at room temperature for 1 min. Then, 200 μl of Solution II was added to the tube and the tube was inverted 6 times and incubated at room temperature for 1 min. Next, 350 μl of Solution III was added to the tubes and gently mixed. The mixture was incubated at room temperature for 1 min then centrifuged at 15000 X G for 1 min. The supernatant was transferred to an EZ-10 column and centrifuged for 2 min. The flow through was discarded and 750 μl Wash Solution was added to column and centrifuged. The wash procedure was repeated and the tube was centrifuged for another 1 min to remove residual Wash Solution. The column was transferred to clean 2 ml tube and 50 μl Elution Buffer was added to the center part of column and allowed to incubate at room temperature for 2 min. The tube was then centrifuged for 2 min. The column was transferred to second 2 ml tube and 20 μl of Elution Buffer was added to center of column and incubated at room temperature for 2 min. The tube was centrifuged for 2 min. Plasmid concentrations were determined by spectrometry (NanoDropND-1000, Nanodrop Technologies, Wilmington, DE) and the plasmid inserts underwent automated sequencing (Davis Sequencing, Davis, CA or Eton, San Diego, CA). Sequences obtained were trimmed, aligned and analyzed for contiguous sequences using Sequencher 4.2 software, then compared to banked sequences in the GenBank database using the NCBI BLAST tool (Altschul et al, 1990). Further alignments of the 18S rDNA and the ITS1-5.8S-ITS2 region sequences were made using ClustalW (EBL-EBI, http://www.ebi.ac.uk/Tools/msa/clustalw2/) with *T. theileri* isolate from cattle (GenBank Accession No. AB007814) as reference sequence.

**Table 4.** *Trypanosoma* 18S ribosomal RNA gene sequences* from elk, WTD and cattle isolates included in the phylogenetic analyses

| Sample | Clone Number | Sequenced ID | Gene Region Cloned | GenBank Accession No. |
|---|---|---|---|---|
| Elk 328 | 20 | MNelk328cl20 | 18S | JX178198 |
| Elk 328 | 21 | MNelk328cl21 | 18S | JX178199 |
| Elk 317 | 3 | MNelk317c3 | 18S | JX178200 |
| Elk 317 | 4 | MNelk317c4 | 18S | JX178201 |
| Elk 142 | 5 | Elk142c5 | ITS | JX178177 |
| Elk 142 | 10 | Elk142c10 | ITS | JX178178 |
| Elk 416 | 8 | Elk416c8 | ITS | JX178179 |
| Elk 328 | 3 | Elk328c3 | ITS | JX178180 |
| WTD A21 | 4 | WTDA21c4 | ITS | JX178174 |
| WTD A21 | 5 | WTDA21c5 | ITS | JX178175 |
| WTD A21 | 6 | WTDA21c6 | ITS | JX178176 |
| Cow 139 | 9 | Cow139c9 | ITS | JX178183 |
| Cow 139 | 10 | Cow139c10 | ITS | JX178184 |
| Cow 139 | 11 | Cow139c11 | ITS | JX178185 |
| Cow 133 | 1 | Cow133c1 | ITS | JX178186 |
| Cow 104 | 1 | Cow104c1 | ITS | JX178187 |
| Cow 104 | 2 | Cow104c2 | ITS | JX178188 |
| Cow 104 | 3 | Cow104c3 | ITS | JX178189 |

*Unpublished data, P.J. Holman

To construct the phylogenetic tree, the cattle and WTD 18S rDNA *Trypanosoma* sequences from this study were aligned with additional unpublished *Trypanosoma* sequences from elk, WTD, and cattle (Table 4) and with *Trypanosoma* 18S rDNA sequences from the GenBank database (Table 5) using ClustalW.

The 18S rRNA gene sequence for *Sterkiella* (formerly *Oxytricha*) *nova* (GenBank Accession No. X03948) served as the outgroup (Table 5). The sequences were trimmed to corresponding aligned regions of approximately 1200 bp. The phylogenetic tree was constructed from the alignment using the neighbor-joining algorithm in PAUP* version 4.0b10 (Wilgenbusch and Swofford, 2003). Molecular distances were estimated using Kimura two-parameter model (Kimura et al., 1980) and robustness tested by 1000 bootstrap replications.

The ITS1-5.8S-ITS2 region sequences were separated into the 18S rDNA (approximately 1500 bp 5' fragment) and the ITS1-5.8S-ITS2 regions (approximately 1200 bp). The two sets of sequences and their additional corresponding sequences (Tables 4 and 6) were trimmed to corresponding matching lengths and aligned by ClustalW. The two resulting alignments were also used to construct phylogenetic trees.

**Table 5.** GenBank 18S ribosomal RNA gene sequences from parasites included in the phylogenetic analyses

| Sequence ID | GenBank Accession # | Parasite Species | Host Species | Reference |
|---|---|---|---|---|
| AJ009164 *T. theileri* | AJ009164 | *Trypanosoma theileri* | cattle | Stevens et al, 1998 |
| AJ009163 *T. theileri* | AJ009163 | *Trypanosoma theileri* | cattle | Stevens et al, 1998 |
| AJ009165 *T. sp* | AJ009165 | *Trypanosoma sp.* | Cervus dama | Stevens et al, 1998 |
| AF065157 *T. rangeli* | AF065157 | *Trypanosoma rangeli* | Not given | Briones et al, unpub |
| AJ012412 *T. leeuwenhoeki* | AJ012412 | *Trypanosoma leeuwenhoeki* | Triatomine bug | Stevens et al, 1999 |
| JN942610 *T. cruzi* | JN942610 | *Trypanosoma cruzi* | Not given | Briones et al, unpub |
| AJ009154 *T. evansi* E110 | AJ009154 | *Trypanosoma evansi* | capybara | Stevens et al, 1998 |
| AJ009142 *T. brucei* TUTRO2509 | AJ009142 | *Trypanosoma brucei* | human | Stevens et al, 1999 |
| AY912268 *T. evansi* KAI | AY912268 | *Trypanosoma evansi* | cattle | Khuchareontaworn et al, unpub |
| AY904050 *T. evansi* buffalo | AY904050 | *Trypanosoma evansi* | buffalo | Khuchareontaworn et al, 2007 |
| AY912269 *T. evansi* SamWTD | AY912269 | *Trypanosoma evansi* | Not given | Khuchareontaworn et al, unpub |
| AJ009153 *T. equiperdum* 818 | AJ009153 | *Trypanosoma equiperdum* | horse | Stevens et al, 1998 |
| AJ009145 *T. congolensi* CAM22b | AJ009145 | *Trypanosoma congolense* | goat | Stevens et al, 1998 |
| AB551921 *T. evansi* | AB551921 | *Trypanosoma evansi* | Dromedary Camel | Amer et al, 2011 |
| AB569250*T. theileri E12* | AB569250 | *Trypanosoma theileri* | cattle | Hatama et al, 2007 |
| AB569249 *t. theileri* E9 | AB569249 | *Trypanosoma theileri* | cattle | Hatama et al, 2007 |
| AB569248 *T.* sp SD1 | AB569248 | *Trypanosoma* sp | Sika WTD | Hatama et al, 2007 |
| AB007814 *T. theileri* | AB007814 | *Trypanosoma theileri* | cattle | Urakawa and Kodama, 1997 |
| AF065157 *T. rangeli* | AF065157 | *Trypanosoma rangeli* | Not given | Briones et al, unpub |
| X03948 *Oxytricha nova** | X03948 | *Sterkiella nova* | | Elwood et al, 1985 |
| AF508771 *Oxytricha nova*** | AF508771 | *Sterkiella nova* | pond | Hewitt et al, 2003 |

*Outgroup for 18S phylogenetic tree from 18S sequence

**Outgroup for 18S phylogenetic tree from ITS1-5.8S-ITS2 sequence

**Table 6.** Ribosomal RNA ITS1-5.8S-ITS2 region sequences from parasites in the GenBank database included in the phylogenetic analysis

| Sequence ID | GenBank Accession # | Parasite Species | Host Species | Reference |
|---|---|---|---|---|
| AB007814 *T. theileri* | AB007814 | *Trypanosoma theileri* | cattle | Urakawa and Kodama, 1997 |
| AB569248 *T.* sp SD1 | AB569248 | *Trypanosoma* sp | Sika WTD | Hatama et al, 2007 |
| AB569250 *T. theileri* E12 | AB569250 | *Trypanosoma theileri* | cattle | Hatama et al, 2007 |
| AB569249 *T. theileri* E9 | AB569249 | *Trypanosoma theileri* | cattle | Hatama et al, 2007 |
| AB551921 *T. evansi** | AB551921 | *Trypanosoma evansi* | Dromedary Camel | Amer et al, 2011 |

*Outgroup for ITS1-5.8S-ITS2 phylogenetic tree

Phylogenetic trees for the 18S rRNA region and the ITS1-5.8S-ITS2 region were constructed from the ClustalW alignments using the neighbor-joining algorithm in PAUP* version 4.0b10 (Swofford, 2002) as above. The 18S rRNA gene sequence for *Sterkiella* (formerly *Oxytricha*) *nova* (GenBank Accession No. X03948) served as the outgroup for the *Trypanosoma* 18S rDNA phylogenetic tree. The ITS1-5.8S-ITS2 sequence from *Trypanosoma evansi* (GenBank Accession No. AB551921) served as the outgroup for the *Trypanosoma* ITS1-5.8S-ITS2 region phylogenetic tree.

## RESULTS

Of the 65 cattle samples and the 336 WTD samples, 23 cattle samples and 173 WTD samples were positive for *Trypanosoma* 18S rDNA by PCR (Table 7). Of the positive cattle samples for the *Trypanosoma* 18S rRNA gene, 2 samples (138 and 2073) were successfully cloned for sequence analysis, and a total of 4 clones were sequenced (Table 8). The fragment sizes ranged from 2000 to 2200 bp. Of the WTD samples positive for the *Trypanosoma* 18S rRNA gene, four (A1, A5, A21, 148) were successfully cloned for sequence analysis, and a total of 7 clones were sequenced (Table 8). The resulting sequences ranged from 2000 to 2200 bp.

A ClustalW alignment of the WTD and cattle *Trypanosoma* 18S rDNA sequences alongside additional sequences from cattle, WTD and elk trypanosomes and *T. theileri* (Genbank) revealed highly conserved sequences among all of the isolates (data not shown; Clustal W alignment, Appendix A). A total of 16 informative sites out of 1154 nucleotide positions were identified (Fig. 2). Two of the cow trypanosome sequences (Cow 2073) obtained in this study hold nucleotides in all 16 positions that match the nucleotides in the *T. theileri* 18S rDNA sequences in GenBank (Accession Nos. AJ009164 and AJ009163; Fig. 2).

Three of the *Trypanosoma* positive cattle samples (Nos. 2, 2095 and 3535) were successfully amplified at the genomic region spanning the *Trypanosoma* ITS1-5.8S-ITS2 region, which included approximately 1500 bp of the 5' end of 18S rRNA gene. The resulting amplicons were cloned and sequenced. The sequenced region was approximately 2200 bp in length and consisted of approximately 1550 bp 18S rDNA, 226 bp ITS1, 169 bp 5.8S and 430 bp ITS2.

| Sequence | Nucleotide Position 42 | 64 | 92 | 506 | 579 | 590 | 810 | 813 | 815 | 816 | 817 | 820 | 843 | 895 | 1130 | 1133 |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| AJ009164 Tth | A | A | A | A | C | G | A | C | - | - | T | C | G | C | T | A |
| AJ009163 Tth | A | A | A | A | C | G | A | C | - | - | T | C | G | C | T | A |
| Cow 2073 cl7 | A | A | A | A | C | G | A | C | - | - | T | C | G | C | T | A |
| Cow 2073 cl | A | A | A | A | C | G | A | C | - | - | T | C | G | C | T | A |
| Cow 138 cl4 | G | T | A | T | A | A | T | T | A | T | C | T | A | T | T | G |
| Cow 138 cl2 | G | T | G | T | A | A | T | T | A | T | C | T | A | T | C | G |
| WTD A21 cl6 | G | T | A | T | C | G | T | T | A | T | T | T | G | C | T | G |
| WTD A5 c11 | G | A | A | T | C | G | T | T | A | T | - | T | G | C | T | G |
| WTD A1 cl1 | G | A | A | T | C | G | T | T | A | T | - | T | G | C | T | G |
| WTD 148 cl4 | G | T | A | T | C | G | T | T | A | T | T | T | G | C | T | G |
| WTD A21 cl4 | G | T | A | T | A | A | T | T | A | T | C | T | A | T | C | G |
| WTD A1 cl4 | G | T | A | T | C | G | T | T | A | T | C | T | G | C | T | G |

**Figure 2.** Informative sites from ClustalW alignment of 18S rRNA region. Yellow represents samples that matched with cattle and green represents samples that matched with WTD. Blue represents samples that did not match with any other samples. The nucleotide positions represent the base pair position starting from the beginning of the 18S sequence (GenBank Accession No AJ009163).

Four of the WTD *Trypanosoma* positive samples (were selected for amplification and cloning of the genomic region spanning the 18S and the ITS1-5.8S-ITS2 region, and two of these (NL15 and A3) yielded a total of 5 clones suitable for sequencing (Table 8). The sequenced region was approximately the same in length and breakdown as the cattle trypanosome sequence.

A ClustalW alignment of the 5' 1500 bp of the 18S rRNA gene and ITS1-5.8S-ITS2 rDNA genomic region showed differences at 346 different base pair positions (Figs. 3 and 4) (full alignment, Appendix B). Most of these differences could be attributed to single-nucleotide polymorphisms (SNP). The ITS1 and ITS2 regions had more variation compared to the 18S and the 5.8S gene regions (which only had SNPs).

**Table 7.** WTD and cattle samples positive by *Trypanosoma* 18S ribosomal DNA PCR. The 18S rRNA gene from the underscored samples were cloned and sequenced. The genomic region spanning part of the 18S rRNA gene and all of the ITS-1-5.8S-ITS2 was cloned and sequenced from the samples that are double underscored.

| **Date (Month/Year)** | **Species** | **Source** | **Animal ID** | **County** | ***T. cervi*** |
|---|---|---|---|---|---|
| May-11 | WTD | SCHUSTER | A1 | Zapata | POS |
| May-11 | WTD | SCHUSTER | A2 | Zapata | POS |
| May-11 | WTD | SCHUSTER | A3 | Zapata | POS |
| May-11 | WTD | SCHUSTER | A4 | Zapata | POS |
| May-11 | WTD | SCHUSTER | A5 | Zapata | POS |
| May-11 | WTD | SCHUSTER | A6 | Zapata | NEG |
| May-11 | WTD | SCHUSTER | A7 | Zapata | POS |
| May-11 | WTD | SCHUSTER | A8 | Zapata | POS |
| May-11 | WTD | SCHUSTER | A9 | Zapata | NEG |
| May-11 | WTD | SCHUSTER | A10 | Zapata | POS |
| May-11 | WTD | SCHUSTER | A11 | Zapata | NEG |
| May-11 | WTD | SCHUSTER | A12 | Zapata | POS |
| May-11 | WTD | SCHUSTER | A13 | Zapata | NEG |
| May-11 | WTD | SCHUSTER | A14 | Zapata | NEG |
| May-11 | WTD | SCHUSTER | A15 | Zapata | POS |
| May-11 | WTD | SCHUSTER | A16 | Zapata | POS |
| May-11 | WTD | SCHUSTER | A17 | Zapata | NEG |
| May-11 | WTD | SCHUSTER | A18 | Zapata | NEG |
| May-11 | WTD | SCHUSTER | A19 | Zapata | NEG |
| May-11 | WTD | SCHUSTER | A20 | Zapata | POS |
| May-11 | WTD | SCHUSTER | A21 | Zapata | POS |
| May-11 | WTD | SCHUSTER | A22 | Zapata | POS |
| May-11 | WTD | SCHUSTER | A23 | Zapata | NEG |
| May-11 | WTD | SCHUSTER | A24 | Zapata | POS |
| May-11 | WTD | SCHUSTER | A25 | Zapata | NEG |
| May-11 | WTD | SCHUSTER | A26 | Zapata | NEG |
| May-11 | WTD | SCHUSTER | A27 | Zapata | NEG |
| May-11 | WTD | SCHUSTER | A28 | Zapata | POS |
| May-11 | WTD | SCHUSTER | A29 | Zapata | POS |
| May-11 | WTD | SCHUSTER | A30 | Zapata | NEG |
| May-11 | WTD | SCHUSTER | A31 | Zapata | POS |
| May-11 | WTD | SCHUSTER | A32 | Zapata | NEG |
| May-11 | WTD | SCHUSTER | A33 | Zapata | NEG |
| May-11 | WTD | SCHUSTER | A34 | Zapata | NEG |
| May-11 | WTD | SCHUSTER | A35 | Zapata | NEG |
| Feb-11 | WTD | SCHUSTER | 147 | Zapata | POS |

**Table 7.** Continued

| Date (Month/Year) | Species | Source | Animal ID | County | *T. cervi* |
|---|---|---|---|---|---|
| Feb-11 | WTD | SCHUSTER | 148 | Zapata | POS |
| Feb-11 | WTD | SCHUSTER | 149 | Zapata | POS |
| Feb-11 | WTD | SCHUSTER | 150 | Zapata | POS |
| Feb-11 | WTD | SCHUSTER | 151 | Zapata | POS |
| Feb-11 | WTD | SCHUSTER | 152 | Zapata | POS |
| Feb-11 | WTD | SCHUSTER | 153 | Zapata | POS |
| Feb-11 | WTD | SCHUSTER | 154 | Zapata | POS |
| Feb-11 | WTD | SCHUSTER | 155 | Zapata | NEG |
| Feb-11 | WTD | SCHUSTER | 156 | Zapata | POS |
| Feb-11 | WTD | SCHUSTER | 157 | Zapata | POS |
| Feb-11 | WTD | SCHUSTER | 158 | Zapata | POS |
| Feb-11 | WTD | SCHUSTER | 159 | Zapata | POS |
| Feb-11 | WTD | SCHUSTER | 160 | Zapata | POS |
| Feb-11 | WTD | SCHUSTER | 161 | Zapata | POS |
| Feb-11 | WTD | SCHUSTER | 162 | Zapata | POS |
| Feb-11 | WTD | SCHUSTER | 163 | Zapata | POS |
| Jul-10 | WTD | SCHUSTER | NL1 | Zapata | POS |
| Jul-10 | WTD | SCHUSTER | NL2 | Zapata | POS |
| Jul-10 | WTD | SCHUSTER | NL3 | Zapata | POS |
| Jul-10 | WTD | SCHUSTER | NL4 | Zapata | POS |
| Jul-10 | WTD | SCHUSTER | NL5 | Zapata | NEG |
| Jul-10 | WTD | SCHUSTER | NL6 | Zapata | POS |
| Jul-10 | WTD | SCHUSTER | NL7 | Zapata | POS |
| Jul-10 | WTD | SCHUSTER | NL8 | Zapata | NEG |
| Jul-10 | WTD | SCHUSTER | NL9 | Zapata | POS |
| Jul-10 | WTD | SCHUSTER | NL10 | Zapata | POS |
| Jul-10 | WTD | SCHUSTER | NL11 | Zapata | POS |
| Jul-10 | WTD | SCHUSTER | NL12 | Zapata | POS |
| Jul-10 | WTD | SCHUSTER | NL13 | Zapata | POS |
| Jul-10 | WTD | SCHUSTER | NL14 | Zapata | POS |
| Jul-10 | WTD | SCHUSTER | NL15 | Zapata | POS |
| Aug 2010 | WTD | SCHUSTER | C1 | Zapata | NEG |
| Aug 2010 | WTD | SCHUSTER | C2 | Zapata | NEG |
| Aug 2010 | WTD | SCHUSTER | C3 | Zapata | POS |
| Aug 2010 | WTD | SCHUSTER | C4 | Zapata | NEG |
| Aug 2010 | WTD | SCHUSTER | C5 | Zapata | NEG |
| Aug 2010 | WTD | SCHUSTER | C6 | Zapata | NEG |

**Table 7.** Continued

| Date (Month/Year) | Species | Source | Animal ID | County | *T. cervi* |
|---|---|---|---|---|---|
| Aug 2010 | WTD | SCHUSTER | C7 | Zapata | NEG |
| Aug 2010 | WTD | SCHUSTER | C8 | Zapata | NEG |
| Aug 2010 | WTD | SCHUSTER | C9 | Zapata | NEG |
| Aug 2010 | WTD | SCHUSTER | C10 | Zapata | NEG |
| Aug 2010 | WTD | SCHUSTER | C11 | Zapata | POS |
| Aug 2010 | WTD | SCHUSTER | C12 | Zapata | NEG |
| Aug 2010 | WTD | SCHUSTER | C13 | Zapata | NEG |
| Aug 2010 | WTD | SCHUSTER | C14 | Zapata | POS |
| Aug 2010 | WTD | SCHUSTER | C15 | Zapata | NEG |
| Aug 2010 | WTD | SCHUSTER | C16 | Zapata | NEG |
| Aug 2010 | WTD | SCHUSTER | C17 | Zapata | NEG |
| Aug 2010 | WTD | SCHUSTER | C18 | Zapata | NEG |
| Aug 2010 | WTD | SCHUSTER | C19 | Zapata | NEG |
| Aug 2010 | WTD | SCHUSTER | C21 | Zapata | POS |
| Aug 2010 | WTD | SCHUSTER | C22 | Zapata | NEG |
| Aug 2010 | WTD | SCHUSTER | C23 | Zapata | NEG |
| Aug 2010 | WTD | SCHUSTER | C25 | Zapata | NEG |
| Aug 2010 | WTD | SCHUSTER | C26 | Zapata | POS |
| Aug 2010 | WTD | SCHUSTER | C27 | Zapata | NEG |
| Aug 2010 | WTD | SCHUSTER | C28 | Zapata | POS |
| Aug 2010 | WTD | SCHUSTER | C30 | Zapata | NEG |
| Aug 2010 | WTD | SCHUSTER | C31 | Zapata | NEG |
| Aug 2010 | WTD | SCHUSTER | C32 | Zapata | POS |
| Aug 2010 | WTD | SCHUSTER | C33 | Zapata | NEG |
| Aug 2010 | WTD | SCHUSTER | C34 | Zapata | NEG |
| Aug 2010 | WTD | SCHUSTER | F1 | Zapata | POS |
| Aug 2010 | WTD | SCHUSTER | SL4 | Zapata | POS |
| Aug 2010 | WTD | SCHUSTER | SL5 | Zapata | NEG |
| Aug 2010 | WTD | SCHUSTER | SL6 | Zapata | NEG |
| Aug 2010 | WTD | SCHUSTER | SL7 | Zapata | NEG |
| Aug 2010 | WTD | SCHUSTER | SL8 | Zapata | NEG |
| Aug 2010 | WTD | SCHUSTER | SL9 | Zapata | NEG |
| Aug 2010 | WTD | SCHUSTER | SL12 | Zapata | NEG |
| Aug 2010 | WTD | SCHUSTER | SL13 | Zapata | NEG |
| Aug 2010 | WTD | SCHUSTER | SL14 | Zapata | NEG |
| Aug 2010 | WTD | SCHUSTER | SL15 | Zapata | NEG |
| Aug 2010 | WTD | SCHUSTER | HF16 | Zapata | POS |

**Table 7.** Continued

| Date (Month/Year) | Species | Source | Animal ID | County | *T. cervi* |
|---|---|---|---|---|---|
| Aug 2010 | WTD | SCHUSTER | HF17 | Zapata | POS |
| Aug 2010 | WTD | SCHUSTER | HF18 | Zapata | POS |
| Aug 2010 | WTD | SCHUSTER | HF19 | Zapata | POS |
| Aug 2010 | WTD | SCHUSTER | HF20 | Zapata | NEG |
| Aug 2010 | WTD | SCHUSTER | HF24 | Zapata | NEG |
| Aug 2010 | WTD | SCHUSTER | HF25 | Zapata | POS |
| Aug 2010 | WTD | SCHUSTER | HF27 | Zapata | POS |
| Aug 2010 | WTD | SCHUSTER | HF28 | Zapata | POS |
| Aug 2010 | WTD | SCHUSTER | HF29 | Zapata | NEG |
| Oct-10 | WTD | SCHUSTER | HF101 | Zapata | POS |
| Oct-10 | WTD | SCHUSTER | HF103 | Zapata | POS |
| Oct-10 | WTD | SCHUSTER | HF104 | Zapata | NEG |
| Oct-10 | WTD | SCHUSTER | HF105 | Zapata | POS |
| Oct-10 | WTD | SCHUSTER | HF106 | Zapata | POS |
| Oct-10 | WTD | SCHUSTER | HF107 | Zapata | POS |
| Oct-10 | WTD | SCHUSTER | HF108 | Zapata | NEG |
| Oct-10 | WTD | SCHUSTER | HF109 | Zapata | POS |
| Oct-10 | WTD | SCHUSTER | HF110 | Zapata | NEG |
| Oct-10 | WTD | SCHUSTER | HF111 | Zapata | POS |
| Oct-10 | WTD | SCHUSTER | HF113 | Zapata | NEG |
| Oct-10 | WTD | SCHUSTER | HF114 | Zapata | POS |
| Oct-10 | WTD | SCHUSTER | HF115 | Zapata | NEG |
| Oct-10 | WTD | SCHUSTER | HF116 | Zapata | NEG |
| Oct-10 | WTD | SCHUSTER | HF117 | Zapata | NEG |
| Oct-10 | WTD | SCHUSTER | HF118 | Zapata | NEG |
| Oct-10 | WTD | SCHUSTER | HF119 | Zapata | POS |
| May-09 | WTD | COOPER | LS1 | La Salle | NEG |
| May-09 | WTD | COOPER | LS2 | La Salle | NEG |
| May-09 | WTD | COOPER | LS3 | La Salle | NEG |
| May-09 | WTD | COOPER | LS4 | La Salle | NEG |
| May-09 | WTD | COOPER | LS5 | La Salle | NEG |
| May-09 | WTD | COOPER | LS6 | La Salle | NEG |
| May-09 | WTD | COOPER | LS7 | La Salle | NEG |
| May-09 | WTD | COOPER | LS8 | La Salle | NEG |
| May-09 | WTD | COOPER | LS9 | La Salle | NEG |
| May-09 | WTD | COOPER | LS10 | La Salle | NEG |
| May-09 | WTD | COOPER | LS11 | La Salle | NEG |

**Table 7.** Continued

| Date (Month/Year) | Species | Source | Animal ID | County | *T. cervi* |
|---|---|---|---|---|---|
| May-09 | WTD | COOPER | LS12 | La Salle | NEG |
| May-09 | WTD | COOPER | LS13 | La Salle | NEG |
| May-09 | WTD | COOPER | LS14 | La Salle | NEG |
| May-09 | WTD | COOPER | LS15 | La Salle | NEG |
| May-09 | WTD | COOPER | LS16 | La Salle | NEG |
| May-09 | WTD | COOPER | LS17 | La Salle | NEG |
| May-09 | WTD | COOPER | LS18 | La Salle | NEG |
| May-09 | WTD | COOPER | LS19 | La Salle | NEG |
| May-09 | WTD | COOPER | LS20 | La Salle | NEG |
| May-09 | WTD | COOPER | LS21 | La Salle | NEG |
| May-09 | WTD | COOPER | LS22 | La Salle | NEG |
| May-09 | WTD | COOPER | LS23 | La Salle | NEG |
| May-09 | WTD | COOPER | LS25 | La Salle | POS |
| May-09 | WTD | COOPER | LS26 | La Salle | NEG |
| May-09 | WTD | COOPER | LS27 | La Salle | NEG |
| May-09 | WTD | COOPER | LS28 | La Salle | NEG |
| May-09 | WTD | COOPER | LS29 | La Salle | NEG |
| May-09 | WTD | COOPER | LS30 | La Salle | NEG |
| May-09 | WTD | COOPER | LS31 | La Salle | NEG |
| May-09 | WTD | COOPER | LS32 | La Salle | POS |
| May-09 | WTD | COOPER | LS33 | La Salle | POS |
| May-09 | WTD | COOPER | LS35 | La Salle | NEG |
| May-09 | WTD | COOPER | LS36 | La Salle | NEG |
| May-09 | WTD | COOPER | LS37 | La Salle | NEG |
| May-09 | WTD | COOPER | LS38 | La Salle | NEG |
| May-09 | WTD | COOPER | LS39 | La Salle | NEG |
| May-09 | WTD | COOPER | LS40 | La Salle | NEG |
| May-09 | WTD | COOPER | LS41 | La Salle | NEG |
| May-09 | WTD | COOPER | LS42 | La Salle | NEG |
| May-09 | WTD | COOPER | LS43 | La Salle | NEG |
| May-09 | WTD | COOPER | LS44 | La Salle | NEG |
| May-09 | WTD | COOPER | LS45 | La Salle | NEG |
| May-09 | WTD | COOPER | LS46 | La Salle | NEG |
| Jan-10 | WTD | COOPER | L1 | Webb | NEG |
| Jan-10 | WTD | COOPER | L2 | Webb | POS |
| Jan-10 | WTD | COOPER | L3 | Webb | POS |
| Jan-10 | WTD | COOPER | L4 | Webb | NEG |

**Table 7.** Continued

| Date (Month/Year) | Species | Source | Animal ID | County | *T. cervi* |
|---|---|---|---|---|---|
| Jan-10 | WTD | COOPER | L5 | Webb | NEG |
| Jan-10 | WTD | COOPER | L6 | Webb | POS |
| Jan-10 | WTD | COOPER | L7 | Webb | NEG |
| Jan-10 | WTD | COOPER | L8 | Webb | NEG |
| Jan-10 | WTD | COOPER | L9 | Webb | NEG |
| Jan-10 | WTD | COOPER | L12 | Webb | POS |
| Jan-10 | WTD | COOPER | L13 | Webb | NEG |
| Jan-10 | WTD | COOPER | L14 | Webb | POS |
| Jan-10 | WTD | COOPER | L15 | Webb | NEG |
| Jan-10 | WTD | COOPER | L16 | Webb | NEG |
| Jan-10 | WTD | COOPER | L17 | Webb | POS |
| Jan-10 | WTD | COOPER | L18 | Webb | POS |
| Jan-10 | WTD | COOPER | L19 | Webb | POS |
| Jan-10 | WTD | COOPER | L20 | Webb | NEG |
| Jan-10 | WTD | COOPER | L21 | Webb | POS |
| Jan-10 | WTD | COOPER | L23 | Webb | NEG |
| Jan-10 | WTD | COOPER | L25 | Webb | POS |
| Jan-10 | WTD | COOPER | L26 | Webb | NEG |
| Jan-10 | WTD | COOPER | L27 | Webb | POS |
| Jan-10 | WTD | COOPER | L28 | Webb | NEG |
| Jan-10 | WTD | COOPER | L30 | Webb | POS |
| Jan-10 | WTD | COOPER | L31 | Webb | POS |
| Jan-10 | WTD | COOPER | L32 | Webb | POS |
| Jan-10 | WTD | COOPER | L33 | Webb | NEG |
| Jan-10 | WTD | COOPER | L34 | Webb | POS |
| Jan-10 | WTD | COOPER | L36 | Webb | NEG |
| Jan-10 | WTD | COOPER | L37 | Webb | POS |
| Jan-10 | WTD | COOPER | L38 | Webb | NEG |
| Jan-10 | WTD | COOPER | L40 | Webb | NEG |
| Aug 2010 | WTD | COOPER | R2 | Zapata | NEG |
| Aug 2010 | WTD | COOPER | R8 | Zapata | NEG |
| Aug 2010 | WTD | COOPER | R11 | Zapata | NEG |
| Aug 2010 | WTD | COOPER | R25 | Zapata | NEG |
| Aug 2010 | WTD | COOPER | R25 | Zapata | NEG |
| Aug 2010 | WTD | COOPER | R31 | Zapata | NEG |
| Aug 2010 | WTD | COOPER | R32 | Zapata | NEG |
| Aug 2010 | WTD | COOPER | R35 | Zapata | NEG |

**Table 7.** Continued

| Date (Month/Year) | Species | Source | Animal ID | County | *T. cervi* |
|---|---|---|---|---|---|
| Aug 2010 | WTD | COOPER | Y15 | Zapata | NEG |
| Jan-10 | WTD | COOPER | W2 | Webb | POS |
| Jan-10 | WTD | COOPER | W3 | Webb | POS |
| Jan-10 | WTD | COOPER | W4 | Webb | NEG |
| Jan-10 | WTD | COOPER | W5 | Webb | NEG |
| Jan-10 | WTD | COOPER | W6 | Webb | NEG |
| Jan-10 | WTD | COOPER | W9 | Webb | NEG |
| Jan-10 | WTD | COOPER | W12 | Webb | NEG |
| Jan-10 | WTD | COOPER | W13 | Webb | NEG |
| Jan-10 | WTD | COOPER | W14 | Webb | NEG |
| Jan-10 | WTD | COOPER | W15 | Webb | NEG |
| Jan-10 | WTD | COOPER | W16 | Webb | NEG |
| Jan-10 | WTD | COOPER | W17 | Webb | NEG |
| Jan-10 | WTD | COOPER | W19 | Webb | NEG |
| Jan-10 | WTD | COOPER | W20 | Webb | NEG |
| Jan-10 | WTD | COOPER | W21 | Webb | NEG |
| Jan-10 | WTD | COOPER | W23 | Webb | NEG |
| Jan-10 | WTD | COOPER | W24 | Webb | NEG |
| Jan-10 | WTD | COOPER | W25 | Webb | NEG |
| Jan-10 | WTD | COOPER | W33 | Webb | NEG |
| Jan-10 | WTD | COOPER | W34 | Webb | NEG |
| Jan-10 | WTD | COOPER | W35 | Webb | NEG |
| Jan-10 | WTD | COOPER | W36 | Webb | POS |
| Jan-10 | WTD | COOPER | W37 | Webb | POS |
| Jan-10 | WTD | COOPER | W38 | Webb | POS |
| Jan-10 | WTD | COOPER | W39 | Webb | POS |
| Jan-10 | WTD | COOPER | W40 | Webb | POS |
| Jan-10 | WTD | COOPER | W41 | Webb | POS |
| Jan-10 | WTD | COOPER | W42 | Webb | POS |
| Jan-10 | WTD | COOPER | W43 | Webb | NEG |
| Jan-10 | WTD | COOPER | W44 | Webb | POS |
| Jan-10 | WTD | COOPER | W45 | Webb | NEG |
| Jan-10 | WTD | COOPER | W46 | Webb | POS |
| Jan-10 | WTD | COOPER | W47 | Webb | POS |
| Mar-10 | Cattle | COOPER | 4 2 | Starr | NEG |
| Mar-10 | Cattle | COOPER | 2 | Starr | POS |
| Mar-10 | Cattle | COOPER | 4 | Starr | NEG |

**Table 7.** Continued

| Date (Month/Year) | Species | Source | Animal ID | County | *T. cervi* |
|---|---|---|---|---|---|
| Mar-10 | Cattle | COOPER | 12 | Starr | NEG |
| Mar-10 | Cattle | COOPER | 24 | Starr | NEG |
| Mar-10 | Cattle | COOPER | 31 | Starr | NEG |
| Mar-10 | Cattle | COOPER | 34 | Starr | NEG |
| Mar-10 | Cattle | COOPER | 36 | Starr | NEG |
| Mar-10 | Cattle | COOPER | 45 | Starr | NEG |
| Mar-10 | Cattle | COOPER | 46 | Starr | NEG |
| Mar-10 | Cattle | COOPER | 47 | Starr | NEG |
| Mar-10 | Cattle | COOPER | 48 | Starr | NEG |
| Mar-10 | Cattle | COOPER | 56 | Starr | NEG |
| Mar-10 | Cattle | COOPER | 60 | Starr | POS |
| Mar-10 | Cattle | COOPER | 63 | Starr | NEG |
| Mar-10 | Cattle | COOPER | 66 | Starr | POS |
| Mar-10 | Cattle | COOPER | 67 | Starr | NEG |
| Mar-10 | Cattle | COOPER | 73 | Starr | NEG |
| Mar-10 | Cattle | COOPER | 95 | Starr | NEG |
| Mar-10 | Cattle | COOPER | 96 | Starr | NEG |
| Mar-10 | Cattle | COOPER | 104 | Starr | POS |
| Mar-10 | Cattle | COOPER | 107 | Starr | POS |
| Mar-10 | Cattle | COOPER | 117 | Starr | NEG |
| Mar-10 | Cattle | COOPER | 128 | Starr | NEG |
| Mar-10 | Cattle | COOPER | 133 | Starr | NEG |
| Mar-10 | Cattle | COOPER | 138 | Starr | POS |
| Mar-10 | Cattle | COOPER | 139 | Starr | POS |
| Mar-10 | Cattle | COOPER | 165 | Starr | NEG |
| Mar-10 | Cattle | COOPER | 169 | Starr | NEG |
| Mar-10 | Cattle | COOPER | 196 | Starr | POS |
| Mar-10 | Cattle | COOPER | 219 | Starr | POS |
| Mar-10 | Cattle | COOPER | 1123 | Starr | NEG |
| Mar-10 | Cattle | COOPER | 2031 | Starr | NEG |
| Mar-10 | Cattle | COOPER | 2048 | Starr | NEG |
| Mar-10 | Cattle | COOPER | 2068 | Starr | NEG |
| Mar-10 | Cattle | COOPER | 2070 | Starr | NEG |
| Mar-10 | Cattle | COOPER | 2073 | Starr | POS |
| Mar-10 | Cattle | COOPER | 2095 | Starr | POS |
| Mar-10 | Cattle | COOPER | 2110 | Starr | NEG |
| Mar-10 | Cattle | COOPER | 2112 | Starr | NEG |

**Table 7.** Continued

| Date (Month/Year) | Species | Source | Animal ID | County | *T. cervi* |
|---|---|---|---|---|---|
| Mar-10 | Cattle | COOPER | 2124 | Starr | POS |
| Mar-10 | Cattle | COOPER | 2125 | Starr | NEG |
| Mar-10 | Cattle | COOPER | 2127 | Starr | POS |
| Mar-10 | Cattle | COOPER | 2166 | Starr | NEG |
| Mar-10 | Cattle | COOPER | 2176 | Starr | NEG |
| Mar-10 | Cattle | COOPER | 2186 | Starr | NEG |
| Mar-10 | Cattle | COOPER | 2201 | Starr | POS |
| Mar-10 | Cattle | COOPER | 3005 | Starr | NEG |
| Mar-10 | Cattle | COOPER | 3006 | Starr | NEG |
| Mar-10 | Cattle | COOPER | 3023 | Starr | NEG |
| Mar-10 | Cattle | COOPER | 3024 | Starr | NEG |
| Mar-10 | Cattle | COOPER | 3026 | Starr | POS |
| Mar-10 | Cattle | COOPER | 3040 | Starr | NEG |
| Mar-10 | Cattle | COOPER | 3071 | Starr | POS |
| Mar-10 | Cattle | COOPER | 3099 | Starr | POS |
| Mar-10 | Cattle | COOPER | 3135 | Starr | POS |
| Mar-10 | Cattle | COOPER | 3520 | Starr | NEG |
| Mar-10 | Cattle | COOPER | 3535 | Starr | POS |
| Mar-10 | Cattle | COOPER | 3539 | Starr | POS |
| Mar-10 | Cattle | COOPER | 3577 | Starr | POS |
| Mar-10 | Cattle | COOPER | 3635 | Starr | POS |
| Mar-10 | Cattle | COOPER | 4253 | Starr | POS |
| Mar-10 | Cattle | COOPER | 9025 | Starr | NEG |
| Mar-10 | Cattle | COOPER | 9082 | Starr | NEG |
| Mar-10 | Cattle | COOPER | 9311 | Starr | NEG |
| Sep-11 | WTD | COOPER | S1 | Starr | POS |
| Sep-11 | WTD | COOPER | S2 | Starr | POS |
| Sep-11 | WTD | COOPER | S3 | Starr | POS |
| Sep-11 | WTD | COOPER | S4 | Starr | POS |
| Sep-11 | WTD | COOPER | S5 | Starr | NEG |
| Sep-11 | WTD | COOPER | S6 | Starr | POS |
| Sep-11 | WTD | COOPER | S7 | Starr | POS |
| Sep-11 | WTD | COOPER | S8 | Starr | POS |
| Sep-11 | WTD | COOPER | S9 | Starr | POS |
| Sep-11 | WTD | COOPER | S11 | Starr | POS |
| Sep-11 | WTD | COOPER | S12 | Starr | POS |
| Sep-11 | WTD | COOPER | S13 | Starr | POS |

**Table 7.** Continued

| Date (Month/Year) | Species | Source | Animal ID | County | *T. cervi* |
|---|---|---|---|---|---|
| Sep-11 | WTD | COOPER | S14 | Starr | POS |
| Sep-11 | WTD | COOPER | S15 | Starr | POS |
| Sep-11 | WTD | COOPER | S17 | Starr | POS |
| Sep-11 | WTD | COOPER | S18 | Starr | POS |
| Sep-11 | WTD | COOPER | S19 | Starr | POS |
| Sep-11 | WTD | COOPER | S20 | Starr | POS |
| Sep-11 | WTD | COOPER | S21 | Starr | POS |
| Sep-11 | WTD | COOPER | S22 | Starr | POS |
| Sep-11 | WTD | COOPER | S23 | Starr | POS |
| Sep-11 | WTD | COOPER | S24 | Starr | POS |
| Sep-11 | WTD | COOPER | S25 | Starr | POS |
| Sep-11 | WTD | COOPER | S26 | Starr | POS |
| Sep-11 | WTD | COOPER | S27 | Starr | POS |
| Sep-11 | WTD | COOPER | S28 | Starr | POS |
| Sep-11 | WTD | COOPER | S29 | Starr | POS |
| Sep-11 | WTD | COOPER | S30 | Starr | POS |
| Sep-11 | WTD | COOPER | S31 | Starr | POS |
| Sep-11 | WTD | COOPER | S32 | Starr | POS |
| Sep-11 | WTD | COOPER | S33 | Starr | NEG |
| Sep-11 | WTD | COOPER | S34 | Starr | POS |
| Sep-11 | WTD | COOPER | S35 | Starr | POS |
| Sep-11 | WTD | COOPER | S36 | Starr | POS |
| Sep-11 | WTD | COOPER | S37 | Starr | POS |
| Sep-11 | WTD | COOPER | S38 | Starr | NEG |
| Sep-11 | WTD | COOPER | S39 | Starr | POS |
| Sep-11 | WTD | COOPER | S40 | Starr | POS |
| Sep-11 | WTD | COOPER | S41 | Starr | POS |
| Sep-11 | WTD | COOPER | S42 | Starr | POS |
| Sep-11 | WTD | COOPER | S43 | Starr | POS |
| Sep-11 | WTD | COOPER | S44 | Starr | POS |
| Sep-11 | WTD | COOPER | S45 | Starr | POS |
| Sep-11 | WTD | COOPER | S46 | Starr | POS |
| Sep-11 | WTD | COOPER | S47 | Starr | POS |
| Sep-11 | WTD | COOPER | S48 | Starr | POS |
| Sep-11 | WTD | COOPER | S49 | Starr | POS |
| Sep-11 | WTD | COOPER | S50 | Starr | POS |
| Sep-11 | WTD | COOPER | S51 | Starr | POS |

**Table 7.** Continued

| Date (Month/Year) | Species | Source | Animal ID | County | *T. cervi* |
|---|---|---|---|---|---|
| Sep-11 | WTD | COOPER | S52 | Starr | POS |
| Sep-11 | WTD | COOPER | S53 | Starr | POS |
| Sep-11 | WTD | COOPER | S54 | Starr | POS |
| Sep-11 | WTD | COOPER | S55 | Starr | POS |
| Sep-11 | WTD | COOPER | S56 | Starr | POS |
| Sep-11 | WTD | COOPER | S57 | Starr | POS |
| Sep-11 | WTD | COOPER | S58 | Starr | POS |
| Sep-11 | WTD | COOPER | S59 | Starr | POS |
| Sep-11 | WTD | COOPER | S60 | Starr | NEG |
| Sep-11 | WTD | COOPER | S61 | Starr | POS |
| Sep-11 | WTD | COOPER | S62 | Starr | POS |
| Sep-11 | WTD | COOPER | S63 | Starr | POS |
| Sep-11 | WTD | COOPER | S64 | Starr | NEG |
| Sep-11 | WTD | COOPER | S65 | Starr | POS |
| Sep-11 | WTD | COOPER | S66 | Starr | POS |
| Sep-11 | WTD | COOPER | S67 | Starr | POS |
| Sep-11 | WTD | COOPER | S68 | Starr | POS |
| Sep-11 | WTD | COOPER | S69 | Starr | POS |
| Sep-11 | WTD | COOPER | S70 | Starr | POS |
| Sep-11 | WTD | COOPER | S71 | Starr | POS |
| Sep-11 | WTD | COOPER | S72 | Starr | NEG |
| Sep-11 | WTD | COOPER | S73 | Starr | POS |
| Sep-11 | WTD | COOPER | S74 | Starr | NEG |
| Sep-11 | WTD | COOPER | S75 | Starr | POS |
| Sep-11 | WTD | COOPER | S76 | Starr | POS |
| Sep-11 | WTD | COOPER | S77 | Starr | POS |
| Sep-11 | WTD | COOPER | S78 | Starr | NEG |
| Sep-11 | WTD | COOPER | S79 | Starr | POS |
| Sep-11 | WTD | COOPER | S80 | Starr | NEG |
| Sep-11 | WTD | COOPER | S81 | Starr | NEG |
| Sep-11 | WTD | COOPER | S82 | Starr | NEG |
| Sep-11 | WTD | COOPER | No # | Starr | POS |

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 Elk142c2        CCATGCCCTTGAGGTCCGTGAACACTCAGAAACAAGAAACACGGGAGCGGTTCCTTCCTG 180
□Elk142c10       CCATGCCCTTGAGGTCCGTGAACACTCAGAAACAAGAAACACGGGAGCGGTTCCTTCCTG 180
□Elk142c5        CCATGCCCTTGAGGTCCGTGAACACTCAGAAACAAGAAACACGGGAGCGGTTCCTTCCTG 180
□Elk328c3        CCATGCCCTTGAGGTCCGTGAACACTCAGAAACAAGAAACACGGGAGCGGTTCCTTCCTG 180
□Elk416c8        CCATGCCCTTGAGGTCCGTGAACACTCAGAAACAAGAAACACGGGAGCGGTTCCTTCCTG 180
□WTDN15c1        CCATGCCCTTGAGGTCCGTGAACACTCAGAAACAAGAAACACGGGAGCGGTTCCTTCCTG 180
□WTDNL15c6       CCATGCCCTTGAGGTCCGTGAACACTCAGAAACAAGAAACACGGGAGCGGTTCCTTCCTG 180
□WTDN15c9        CCATGCCCTTGAGGTCCGTGAACACTCAGAAACAAGAAACACGGGAGCGGTTCCTTCCTG 180
□WTDA21c4        CCATGCCCTTGAGGTCCGTGAACACTCAGAAACAAGAAACACGGGAGCGGTTCCTTCCTG 180
□WTDA21c5        CCATGCCCTTGAGGTCCGTGAACACTCAGAAACAAGAAACACGGGAGCGGTTCCTTCCTG 180
□WTDA21c6        CCATGCCCTTGAGGTCCGTGAACACTCAGAAACAAGAAACACGGGAGCGGTTCCTTCCTG 180
□Cow139c10       CCATGCCCTTGAGGTCCGTGAACAATCAGAAACAAAAAACACGGGAGCGGTTCCTTCCTG 180
□Cow139c11       CCATGCCCTTGAGGTCCGTGAACAATCAGAAACAAAAAACACGGGAGCGGTTCCTTCCTG 180
□Cow2c4          CCATGCCCTTGAGGTCCGTGAACAATCAGAAACAAAAAACACGGGAGCGGTTCCTTCCTG 180
□Cow133c1        CCATGCCCTTGAGGTCCGTGAACAATCAGAAACAAAAAACACGGGAGCGGTTCCTTCCTG 180
□Cow139c9        CCATGCCCTTGAGGTCCGTGAACAATCAGAAACAAAAAACACGGGAGCGGTTCCTTCCTG 180
□Cow3535c3       CCATGCCCTTGAGGTCCGTGAACAATCAGAAACAAAAAACACGGGAGCGGTTCCTTCCTG 180
□Cow3535c6       CCATGCCCTTGAGGTCCGTGAACAATCAGAAACAAAAAACACGGGAGCGGTTCCTTCCTG 180
□Cow2c7          CCATGCCCTTGAGGTCCGTGAACAATCAGAAACAAAAAACACGGGAGCGGTTCCTTCCTG 180
□Cow2c10         CCATGCCCTTGAGGTCCGTGAACAATCAGAAACAAAAAACACGGGAGCGGTTCCTTCCTG 180
□AB007814Tth     CCATGCCCTTGAGGTCCGTGAACAATCAGAAACAAAAAACACGGGAGCGGTTCCTTCCTG 180
□Cow104c2        CCATGCCCTTGAGGTCCGTGAACAATCAGAAACAAAAAACACGGGAGCGGTTCCTTCCTG 180
□Cow104c3        CCATGCCCTTGAGGTCCGTGAACAATCAGAAACAAAAAACACGGGAGCGGTTCCTTCCTG 180
□Cow104c1        CCATGCCCTTGAGGTCCGTGAATAATCAGAAACAAAAAACACGGGAGCGGTTCCTTCCTG 180
□Cow2095c4       CCATGCCCTTGAGGTCCGTGAACAATCAGAAACAAAAAACACGGGAGCGGTTCCTTCCTG 180
□Cow2095c9       CCATGCCCTTGAGGTCCGTGAACAATCAGAAACAAAAAACACGGGAGCGGTTCCTTCCTG 180
□Cow2095c8       CCATGCCCTTGAGGTCCGTGAACAATCAGAAACAAAAAACACGGGAGCGGTTCCTTCCTG 180
□WTDA3c9.1       CCATGCCCTTGAGGTCCGTGAACACTCAGAAACAAGAAACACGGGAGCGGTTCCTTCCTG 180
□WTDA3c9.4       CCATGCCCTTGAGGTCCGTGAACACTCAGAAACAAGAAACACGGGAGCGGTTCCTTCCTG 180
□                ********************** * ********** ************************
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**Figure 3.** ClustalW alignment. Partial 18S rRNA gene region and rRNA ITS1, 5.8S, ITS2 region are shown with nucleotide position number indicated at the right. Matching nucleotides in all sequences are shown by *. WTD trypanosome specific nucleotides are indicated in blue; cattle in red. Gaps (deletions) are indicated by a dash (-). Omitted data is indicated by //.

```
//
Elk142c2          ACAAGCGTCTGGGTGTTTTCCCATTTTTGGGGCACCCGTCGCCTTTGCGGGAAATCCGTG 420
Elk142c10         ACAAGCGTCTGGGTCTTTTCCCATTTTTGGGGCACCCGTCGCCTTTGCGGGAAATCCGTG 420
Elk142c5          ACAAGCGTCTGGGTGTTTTCCCATTTTTGGGGCACCCGTCGCCTTTGCGGGAAATCCGTG 420
Elk328c3          ACAAGCGTCTGGGTGTTTTCCCATTTTTGGGGCACCCGTCGCCTTTGCGGGAAATCCGTG 420
Elk416c8          ACAAGCGTCTGGGTGTTTTCCCATTTTTGGGGCACCCGTCGCCTTTGCGGGAAATCCGTG 420
WTDN15c1          ACAAGCGTCTGGGTGTTTTCCCATTTT-GGGGCACCCGTCGCCTTTGCGGGAAATCCGTG 419
WTDNL15c6         ACAAGCGTCTGGGTGTTTTCCCATTTT-GGGGCACCCGTCGCCTTTGCGGGAAATCCGTG 419
WTDN15c9          ACAAGCGTCTGGGTGTTTTCCCATTTT-GGGGCACCCGTCGCCTTTGCGGGAAATCCGTG 419
WTDA21c4          ACAAGCGTCTGGGTGTTTTCCCATTTT-GGGGCACCCGTCGCCTTTGCGGGAAATCCGTG 419
WTDA21c5          ACAAGCGTCTGGGTGTTTTCCCATTTTTGGGGCACCCGTCGCCTTTGCGGGAAATCCGTG 420
WTDA21c6          ACAAGCGTCTGGGTGTTTTCCCATTTT-GGGGCACCCGTCGCCTTTGCGGGAAATCCGTG 419
Cow139c10         ACAAGCGTCTGGGTGTTTTCCCATCTTTGGGGCACCCGTCGCCTTTGCGAGAAATCCGTG 420
Cow139c11         ACAAGCGTCTGGGTGTTTTCCCATCTTTGGGGCACCCGTCGCCTTTGCGAGAAATCCGTG 420
Cow2c4            ACAAGCGTCTGGGTGTTTTCCCATCTTTGGGGCACCCGTCGCCTTTGCGAGAAATCCGTG 420
Cow133c1          ACAAGCGTCTGGGTGTTTTCCCATCTTTGGGGCACCCGTCGCCTTTGCGAGAAATCCGTG 420
Cow139c9          ACAAGCGTCTGGGTGTTTTCCCATCTTTGGGGCACCCGTCGCCTTTGCGAGAAACCCGTG 420
Cow3535c3         ACAAGCGTCTGGGTGTTTTCCCATCTTTGGGGCACCCGTCGCCTTTGCGAGAAATCCGTG 420
Cow3535c6         ACAAGCGTCTGGGTGTTTTCCCATCTTTGGGGCACCCGTCGCCTTTGCGAGAAATCCGTG 420
Cow2c7            ACAAGCGTCTGGGTGTTTTCCCATCTTTGGGGCACCCGTCGCCTTTGTGAGAAATCCGTG 420
Cow2c10           ACAAGCGTCTGGGTGTTTTCCCATCTTTGGGGCACCCGTCGCCTTTGCGAGAAATCCGTG 420
AB007814Tth       ACAAGCGTCTGGGTGTTTTCCCATCTTTGGGGCACCCGTCGCCTTTGCGAGAAATCCGTG 420
Cow104c2          ACAAGCGTCTGGGTGTTTTCCCATCTTTGGGGCACCCGTCGCCTTTGCGAGAAATCCGTG 420
Cow104c3          ACAAGCGTCTGGGTGTTTTCCCATCTTTGGGGCACCCGTCGCCTTTGCGAGAAATCCGTG 420
Cow104c1          ACAAGCGTCTGGGTGTTTTCCCATCTTTGGGGCACCCGTCGCCTTTGCGAGAAATCCGTG 420
Cow2095c4         ACAAGCGTCTGGGTGTTTTCCCATCTTTGGGGCACCCGTCGCCTTTGCGAGAAATCCGTG 420
Cow2095c9         ACAAGCGTCTGGGTGTTTTCCCATCTTTGGGGCACCCGTCGCCTTTGCGAGAAATCCGTG 420
Cow2095c8         ACAAGCGTCTGGGTGTTTTCCCATCTTTGGGGCACCCGTCGCCTTTGCGAGAAATCCGTG 420
WTDA3c9.1         ACAAGCGTCTGGGTGATTCCCC--TCTTGGGGCGCCCGTCGCCTTTGCGGGAAATCCGTG 418
WTDA3c9.4         ACAAGCGTCTGGGTGATTCCCC--TCTTGGGGCGCCCGTCGCCTTTGCGGGAAATCCGTG 418
                  **************  ** ***    * ***** ************* * **** *****
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**Figure 3** continued

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 Elk142c2          TCGGTGTTGATGAGCTTCGGCCCATCTCTCCGGCGCCTTCCCGTGACTCACGGCATCCAG 480
 Elk142c10         TCGGTGTTGATGAGCTTCGGCCCATCTCTCCGGCGCCTTCCCGTGACTCACGGCATCCAG 480
 Elk142c5          TCGGTGTTGATGAGCTTCGGCCCATCTCTCCGGCGCCTTCCCGTGACTCACGGCATCCAG 480
 Elk328c3          TCGGTGTTGATGAGCTTCGGCCCATCTCTCCGGCGCCTTCCCGTGACTCACGGCATCCAG 480
 Elk416c8          TCGGTGTTGATGAGCTTCGGCCCATCTCTCCGGCGCCTTCCCGTGACTCACGGCATCCAG 480
 WTDN15c1          TCGGTGTTGATGAGCTTCGGCCCATCTCTCCGGCGCCTTCCCGTGACTCACGGCATCCAG 479
 WTDNL15c6         TCGGTGTTGATGAGCTTCGGCCCATCTCTCCGGCGCCTTCCCGTGACTCACGGCATCCAG 479
 WTDN15c9          TCGGTGTTGATGAGCTTCGGCCCATCTCTCCGGCGCCTTCCCGTGACTCACGGCATCCAG 479
 WTDA21c4          TCGGTGTTGATGAGCTTCGGCCCATCTCTCCGGCGCCTTCCCGTGACTCACGGCATCCAG 479
 WTDA21c5          TCGGTGTTGATGAGCTTCGGCCCATCTCTCCGGCGCCTTCCCGTGACTCACGGCATCCAA 480
 WTDA21c6          TCGGCGTTGATGAGCTTCGGCCCATCTCTCCGGCGCCTTCCCGTGACTCACGGCATCCAG 479
 Cow139c10         TCGGTGTTGATGAGCTTCGGCCCATCTCTCCGGCGCCTTCTCGTGACTCACGGCATCCAG 480
 Cow139c11         TCGGTGTTGATGAGCTTCGGCCCATCTCTCCGGCGCCTTCTCGTGACTCACGGCATCCAG 480
 Cow2c4            TCGGTGTTGATGAGCTTCGGCCCATCTCTCCGGCGCCTTCTCGTGACTCACGGCATCCAG 480
 Cow133c1          TCGGTGTTGATGAGCTTCGGCCCATCTCTCCGGCGCCTTCTCGTGACTCACGGCATCCAG 480
 Cow139c9          TCGGTGTTGATGAGCTTCGGCCCATCTCTCCGGCGCCTTCTCGTGACTCACGGCATCCAG 480
 Cow3535c3         TCGGTGTTGATGAGCTTCGGCCCATCTCTCCGGCGCCTTCTCGTGACTCACGGCATCCAG 480
 Cow3535c6         TCGGTGTTGATGAGCTTCGGCCCATCTCTCCGGCGCCTTCTCGTGACTCACGGCATCCAG 480
 Cow2c7            TCGGTGTTGATGAGCTTCGGCCCATCTCTCCGGCGCCTTCTCGTGACTCACGGCATCCAG 480
 Cow2c10           TCGGTGTTGATGAGCTTCGGCCCATCTCTCCGGCGCCTTCTCGTGACTCACGGCATCCAG 480
 AB007814Tth       TCGGTGTTGATGAGCTTCGGCCCATCTCTCCGGCGCCTTCTCGTGACTCACGGCATCCAG 480
 Cow104c2          TCGGTGTTGATGAGCTTCGGCCCATCTCTCCGGCGCCTTCTCGTGACTCACGGCATCCAG 480
 Cow104c3          TCGGTGTTGATGAGCTTCGGCCCATCTCTCCGGCGCCTTCTCGTGACTCACGGCATCCAG 480
 Cow104c1          TCGGTGTTGATGAGCTTCGGCCCATCTCTCCGGCGCCTTCTCGTGACTCACGGCATCCAG 480
 Cow2095c4         TCGGTGTTGATGAGCTTCGGCCCATCTCTCCGGCGCCTTCTCGTGACTCACGGCATCCAG 480
 Cow2095c9         TCGGTGTTGATGAGCTTCGGCCCATCTCTCCGGCGCCTTCTCGTGACTCACGGCATCCAG 480
 Cow2095c8         TCGGTGTTGATGAGCTTCGGCCCATCTCTCCGGCGCCTTCTCGTGACTCACGGCATCCAG 480
 WTDA3c9.1         TCGGTGTTGATGAGCTTCGGCCCATCTCTCCGGCGCCTTCCCGTGACTCACGGCATCCAG 478
 WTDA3c9.4         TCGGTGTTGATGAGCTTCGGCCCATCTCTCCGGCGCCTTCCCGTGACTCACGGCATCCAG 478
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**Figure 3** continued

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//
Elk142c2          --ATATGTACCGCGGGGTGGAA--TAGTAT---TTATGTATATATTATTATATACAT-AT 1665
Elk142c10         --ATATGTACCGCGGGGTGGAA--TAGTAT---TTATGTATATATTATTACATACAT-AA 1667
Elk142c5          --ATATGTACCGCGGGGTGGAA--TA------------------------TACATAT-AT 1643
Elk328c3          -TATATGTACCGCGGGGTGGAA--TAGTAT---T-GTGTATAT----TTATATACAT-AT 1664
Elk416c8          -TATATGTACCGCGGGGTGGAA--TAGTAT---T-GTGTATAT----TTATATACAT-AT 1664
WTDN15c1          ATATATGTACCGCGGGGTGGAAAATA-TAT---T-GTGTATAT----TTATATGCAT-AT 1667
WTDNL15c6         -TATATGTACCGCGGGGTGGAAAATA-TAT---T-GTGTATAT----TTATATGCAT-AT 1664
WTDN15c9          -TATATGTACCGCGGGGTGGAAAATA-TAT---T-GTGTATAT----TTATATGCAT-AT 1664
WTDA21c4          ATATATGTACCGCGGGGTGGAAAATAGTAT---T-GTGTATAT----TTATATGCAT-AT 1669
WTDA21c5          ATATATGTACCGCGGGGTGGAAAATAGTAT---T-GTGTATAT----TTATATGCAT-AT 1668
WTDA21c6          ATATATGTACCGCGGGGTGGAA--TAATAT---T-GTGTATAT----TTATATACAT-AT 1664
Cow139c10         ---TATGTACCGCGGGGTGGAA--TAATATATTTTATGTATGTA----TATAT--AC-AT 1664
Cow139c11         ---TATGTACCGCGGGGTGGAA--TAATATATTTTATGTATGTA----TATAT--AC-AT 1664
Cow2c4            ---TATGTACCGCGGGGTGGAA--TAATATATTTTATGTATGTA----TATAT--AC-AT 1664
Cow133c1          ---TATGTACCGCGGGGTGGAA--TAATATATTTTATGTATGTA----TATAT--AC-AT 1664
Cow139c9          ---TATGTACCGCGGGGTGGAA--TAATATATTTTATGTATGTA----TATAT--AC-AT 1664
Cow3535c3         ---TATGTACCGCGGGGTGGAA--TAATATATTTTATGTATGTA----TATAT--AC-AT 1664
Cow3535c6         ---TATGTACCGCGGGGTGGAA--TAATATATTTTATGTATGTA----TATAT--AC-AT 1664
Cow2c7            ---TATGTACCGCGGGGTGGAA--TAATATATTTTATGTATGTA----TATAT--AC-AT 1664
Cow2c10           ---TATGTACCGCGGGGTGGAA--TAATATATTTTATGTATGTA----TATAT--AC-AT 1664
AB007814Tth       ---TATGTACCGCGGGGTGGAA--TAATATATTTTATGTATGTA----TATAT--AC-AT 1664
Cow104c2          ---TATGTACCGCGGGGTGGAA--TAATATATTTTATGTATGTA----TATATGTAC-AT 1665
Cow104c3          ---TATGTACCGCGGGGTGGAA--TAATATATTTTATGTATGTA----TATATGTAC-AT 1665
Cow104c1          ---TATGTACCGCGGGGTGGAA--TAATATATTTTATGTATGTA----TATATGTAC-AT 1665
Cow2095c4         ---TATGTACCGCGGGGTGGAA--TAATATATTTTATGTATATG----TATATATAC-AT 1665
Cow2095c9         ---TATGTACCGCGGGGTGGAA--TAATATATTTTATGTATATG----TATATATAC-AT 1665
Cow2095c8         ---TATGTACCGCGGGGTGGAA--TAATATATTTTATGTATATG----TATATATAC-AT 1665
WTDA3c9.1         ---TAT-----------TATAA--TAATATG---CATGTGTAC-----CGCGGGGAGGAA 1650
WTDA3c9.4         ---TAT-----------TATAA--TAATATG---CATGTGTAC-----CGCGGGGAGGAA 1650
                     ***           *  **  **                             *  *
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**Figure 3** continued

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Elk142c2         ATATGTA--TTTCCTCCTTCGCACAGATATAACACATATTGCATTT-CGTCTGTTGC--T 1720
Elk142c10        ATATGTA--TTTCCTCCTTCGCACAGATATAACACATATTGCATTTTCGTCTGTTGC--T 1723
Elk142c5         ATATGTA--TTTCCTCCTTCGCACAGATATAACACATATTGCATTTTCGTCTGTTGC--T 1699
Elk328c3         ATATATATATTTCCTCCTTCGCACAGATATATTACATATTGCATTT-TGTCTGTTGTGTT 1723
Elk416c8         ATATATATATTTCCTCCTTCGCACAGATATATTACATATTGCATTT-TGTCTGTTGTGTT 1723
WTDN15c1         ATATATATATTTCCTCCTTCGCACAGATATATTACATATTGCATTT-TGTCTGTTGTGTT 1726
WTDNL15c6        ATATATA-ATTTCCTCCTTCGCACAGATATATTACATATTGCATTT-TGTCTGTTGTGTT 1722
WTDN15c9         ATATATA--TTTCCTCCTTCGCACAGATATATTACATATTGCATTT-TGTCTGTTGTGTT 1721
WTDA21c4         ATATATA--TTTCCTCCTTCGCACAGATATATTACATATTGCATTT-TGTCTGTTGTGTT 1726
WTDA21c5         ATATATA--TTTCCTCCTTCGCACAGATATATTACATATTGCATTT-TGTCTGTTGTGTT 1725
WTDA21c6         ATATATG--TTTCCTCCTTCGCACAGATATATTACATATTGCATTT-TGTCTGTTGTGTT 1721
Cow139c10        ATGTATA--TTTCCTCCTTCGCACAGATGTATTACATATTGCATTT-TGTCTGTTGT--T 1719
Cow139c11        ATGTATA--TTTCCTCCTTCGCACAGATGTATTACATATTGCATTT-TGTCTGTTGT--T 1719
Cow2c4           ATGTATA--TTTCCTCCTTCGCACAGATGTATTACATATTGCATTT-TGTCTGTTGT--T 1719
Cow133c1         ATGTATA--TTTCCTCCTTCGCACAGATGTATTACATATTGCATTT-TGTCTGTTGT--T 1719
Cow139c9         ATGTATA--TTTCCTCCTTCGCACAGATGTATTACATATTGCATTT-TGTCTGTTGT--T 1719
Cow3535c3        ATGTATA--TTTCCTCCTTCGCACAGATGTATTACATATTGCATTT-TGTCTGTTGT--T 1719
Cow3535c6        ATGTATA--TTTCCTCCTTCGCACAGATGTATTACATATTGCATTT-TGTCTGTTGT--T 1719
Cow2c7           ATGTATA--TTTCCTCCTTCGCACAGATGTATTACATATTGCATTT-TGTCTGTTGT--T 1719
Cow2c10          ATGTATA--TTTCCTCCTTCGCACAGATGTATTACATATTGCATTT-TGTCTGTTGT--T 1719
AB007814Tth      ATGTATA--TTTCCTCCTTCGCACAGATGTATTACATATTGCATTT-TGTCTGTTGT--T 1719
Cow104c2         ATGTATA--TTTCCTCCTTCGCACAGATGTATTACATATTGCATTT-TGTCTGTTGT--T 1720
Cow104c3         ATGTATA--TTTCCTCCTTCGCACAGATGTATTACATATTGCATTT-TGTCTGTTGT--T 1720
Cow104c1         ATGTATA--TTTCCTCCTTCGCACAGATGTATTACATATTGCATTT-TGTCTGTTGT--T 1720
Cow2095c4        ATGTATG--TTTCCTCCTTCGCACAGATGTATTACATATTGCATTT-TGTCTGTTGT--T 1720
Cow2095c9        ATGTATA--TTTCCTCCTTCGCACAGATGTATTACATATTGCATTT-TGTCTGTTGT--T 1720
Cow2095c8        ATGTATA--TTTCCTCCTTCGCACAGATGTATTACATATTGCATTT-TGTCTGTTGT--T 1720
WTDA3c9.1        ATGTCAA--TTTCTTCCTTCGCACAGAT--ATTATAT-CTGTGTTT---TCTTTTGCGCC 1702
WTDA3c9.4        ATGTCAA--TTTCTTCCTTCGCACAGAT--ATTATAT-CTGTGTTT---TCTTTTGCGCC 1702
                 ** *     **** **************  *  * **  **  ***   *** ***
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**Figure 3** continued

```
Elk142c2          GTGTGTGGGTGT-----ATAACTCATACACG-GCCTCAGACAG-GTGCAATAACAAAAAA 1773
Elk142c10         GTGTGTGGGTGT-----ATAACTCATACACG-GCCTCAGACAG-GTGCAATAACAAAAAA 1776
Elk142c5          GTGTGTGGGTGT-----ATAACTCATACACG-GCCTCAGACAG-GTGCAATAACAAAAAA 1752
Elk328c3          GTGTGTGGGTGT-----ATAACCCATACACA-GCCGCAGACAGAGTGCAATAGCAAAAAA 1777
Elk416c8          GTGTGTGGGTGT-----ATAACCCATACACA-GCCGCAGACAGAGTGCAATAGCAAAAAA 1777
WTDN15c1          GTGTGTGGGTGTT----ATAACTCATACACA-GCTGCAGACAGAGTGCAATAGCAAAAAA 1781
WTDNL15c6         GTGTGTGGGTGTT----ATAACTCATACACA-GCTGCAGACAGAGTGCAATAGCAAAAAA 1777
WTDN15c9          GTGTGTGGGTGTT----ATAACTCATACACA-GCTGCAGACAGAGTGCAATAGCAAAAAA 1776
WTDA21c4          GTGTGTGGGTGTT----ATAACTCATACACA-GCCGCAGACAGAGTGCAATAGCAAAAAA 1781
WTDA21c5          GTGTGTGGGTGTT----ATAACTCATACACA-GCCGCAGACAGAGTGCAATAGCAAAAAA 1780
WTDA21c6          GTGTGTGGGTGT-----ATAACTCATGCACA-GCCGCAGACAGAGTGCAATAGCAAAAAA 1775
Cow139c10         GTGTGTGGGTGTAT---ATCTCTCATGCACA-GCCTCAGACAG--TGCAATAACAAAAAA 1773
Cow139c11         GTGTGTGGGTGTAT---ATCTCTCATGCACA-GCCTCAGACAG--TGCAATAACAAAAAA 1773
Cow2c4            GTGTGTGGGTGTAT---ATCTCTCACGCACA-GCCTCAGACAG--TGCAATAACAAAAAA 1773
Cow133c1          GTGTGTGGGTGTAT---ATCTCTCATGCACA-GCCTCAGACAG--TGCAATAACAAAAAA 1773
Cow139c9          GTGTGTGGGTGTAT---ATCTCTCATGCACA-GCCTCAGACAG--TGCAATAACAAAAAA 1773
Cow3535c3         GTGTGTGGGTGTAT---ATCTCTCATGCACA-GCCTCAGACAG--TGCAATAACAAAAAA 1773
Cow3535c6         GCGTGTGGGTGTAT---ATCTCTCATGCACA-GCCTCAGACAG--TGCAATAACAAAAAA 1773
Cow2c7            GTGTGTGGGTGTAT---ATCTCTCATGCACA-GCCTCAGACAG--TGCAATAACAAAAAA 1773
Cow2c10           GTGTGTGGGTGTAT---ATCTCTCATGCACA-GCCTCAGACAG--TGCAATAACAAAAAA 1773
AB007814Tth       GTGTGTGGGTGTAT---ATCTCTCATGCACA-GCCTCAGACAG--TGCAATAACAAAAAA 1773
Cow104c2          GTGTGTGGGTGTAT---ATCTCTCATGCACA-GCCTCAGACAG--TGCAATAACAAAAAA 1774
Cow104c3          GTGTGTGGGTGTAT---ATCTCTCATGCACA-GCCTCAGACAG--TGCAATAACAAAAAA 1774
Cow104c1          GTGTGTGGGTGTAT---ATCTCTCATGCACA-GCCTCAGACAG--TGCAATAACAAAAAA 1774
Cow2095c4         GTGTGTGGGTGTATTATATCTCTCATGCACA-GCCTCAGACAG--TGCAATAACAAAAAA 1777
Cow2095c9         GTGTGTGGGTGTATTATATCTCTCATGCACA-GCCTCAGACAG--TGCAATAACAAAAAA 1777
Cow2095c8         GTGTGTGGGTGTATTATATCTCTCATGCACA-GCCTCAGACAG--TGCAATAACAAAAAA 1777
WTDA3c9.1         ATACCTAGTAGTC----ATA-CTTAGGTGTGTGATGCAAA-GGAAAACACAAACAAAAAA 1756
WTDA3c9.4         ATACCTAGTAGTC----ATA-CTTAGGTGTGTGATGCAAA-GGAAAACACAAACAAAAAA 1756
                       * *  **     **  *  *       *   ** *  *    **  * *******
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**Figure 3** continued

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Elk142c2        AA-CTCATGCCGCTTGACTTTCTCCACATAATACTTTATT---ATGTG-TGATTGTTGAG 1828
Elk142c10       A--CTCATGCCGCTTGACTTTCTCCACATAATACTTTATT---ATGTG-TGATTGTTGAG 1830
Elk142c5        AA-CTCATGCCGCTTGACTCTCTCCACATAATACTTTATT---ATGTG-TGATTGTTGAG 1807
Elk328c3        AA-CTCATGCCGCTTGACTCTC--CACATAAT----TAT------GTG-TGATTGTTGAG 1823
Elk416c8        AA-CTCATGCCGCTTGACTCTC--CACATAAT----TAT------GTG-TGATTGTTGAG 1823
WTDN15c1        AA-CTCATGCCGCTTGACTCTC--CACATAAT----TAT------GTG-TGATTGTTGAG 1827
WTDNL15c6       AA-CTCATGCCGCTTGACTCTC--CACATAAT----TAT------GTG-TGATTGTTGAG 1823
WTDN15c9        AA-CTCATGCCGCTTGACTCTC--CACATAAT----TAT------GTG-TGATTGTTGAG 1822
WTDA21c4        AA-CTCATGCCGCTTGACTCTC--CACATAAT----TAT------GTG-TGATTGTTGAG 1827
WTDA21c5        AA-CTCATGCCGCTTGACTCTC--CACATAAT----TAT------GTG-TGATTGTTGAG 1826
WTDA21c6        AAACTCATGCCGCTTGACTCTCTCCACATAAT----TATTGTTATGTG-TGATCGTTGAG 1830
Cow139c10       AA-CTCATGCCGCTTGACTCTCTTCACATAATAATTAATTATTATATGGTGATTGTTGAG 1832
Cow139c11       AAACTCATGCCGCTTGACTCTCTTCACATAATAATTAATTATTATATGGTGATTGTTGAG 1833
Cow2c4          AAACTCATGCCGCTTGACTCTCTTCACATAATAATTAATTATTATATGGTGATTGTTGAG 1833
Cow133c1        AA-CTCATGCCGCTTGACTCTCTTCACATAATAATTAATTATTATATGGTGATTGTTGAG 1832
Cow139c9        AA-CTCATGCCGCTTGACTCTCTTCACATAATAATTAATTATTATATGGTGATTGTTGAG 1832
Cow3535c3       AA-CTCATGCCGCTTGACTCTCTTCACATAATAATTAATTATTATATGGTGATTGTTGAG 1832
Cow3535c6       AA-CTCATGCCGCTTGACTCTCTTCACATAATAATTAATTATTATATGGTGATTGTTGAG 1832
Cow2c7          AA-CTCATGCCGCTTGACTCTCTTCACATAATAATTAATTATTATATGGTGATTGTTGAG 1832
Cow2c10         AA-CTCATGCCGCTTGACTCTCTTCACATAATAATTAATTATTATATGGTGATTGTTGAG 1832
AB007814Tth     AA-CTCATGCCGCTTGACTCTCTTCACATAATAATTAATTATTATATGGTGATTGTTGAG 1832
Cow104c2        AA-CTCATGCCGCTTGACTTTCTTCACATAATAATTAATTATTATGTGGTGATTGTTGAG 1833
Cow104c3        AA-CTCATGCCGCTTGACTTTCTTCACATAATAATTAATTATTATGTGGTGATTGTTGAG 1833
Cow104c1        AA-CTCATGCCGCTTGACTTTCTTCACATAATAATTAATTATTATGTGGTGATTGTTGAG 1833
Cow2095c4       AA-CTCATGCCGCTTGACTTTCTTCACATAATAATTAATTATTATGTGGTGATTGTTGAG 1836
Cow2095c9       AA-CTCATGCCGCTTGACTCTCTTCACATAATAATTAATTATTATGTGGTGATTGTTGAG 1836
Cow2095c8       AA-CTCATGCCGCTTGACTCTCTTCACATAATAATTAATTATTATGTGGTGATTGTTGAG 1836
WTDA3c9.1       AA-CTCATGCCGCTTGACTCTCCCACATATTATTTTAT----GTGTG-TGATTGTTGAG 1810
WTDA3c9.4       AA-CTCATGCCGCTTGACTCTCCCACATATTATTTTAT----GTGTG-TGATTGTTGAG 1810
                *  ***************  **  ****** *     **       ** **** ******
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**Figure 3** continued

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//
 Elk142c2          CAGAGAGCCCTTGGGGATACGGATGAAACACATTCTTTCAGCGTGCGTTTACTCGCATGCA 2128
 Elk142c10         CAGAGAGCCCTTGGGGATACGGATGAAACACACTCTTTCAGCGTGCGTTTACTCGCATGCA 2130
 Elk142c5          CAGAGAGCCCTTGGGGATACGGATGAAACACACTCTTTCAGCGTGCGTTTACTCGCATGCA 2107
 Elk328c3          CAGAGAGCCCTTGGGGATACGGATGAAACACACTCTTTCAGCGCGCGTTTACTCGCATGCA 2123
 Elk416c8          CAGAGAGCCCTTGGGGATACGGATGAAACACACTCTTTCAGCGCGCGTTTACTCGCATGCA 2123
 WTDN15c1          CAGAGAGCCCTTGGGGATACGGATGAAACACACTCTTTCAGCGCGCGTTTACTCGCATGCA 2125
 WTDNL15c6         CAGAGAGCCCTTGGGGATACGGATGAAACACACTCTTTCAGCGCGCGTTTACTCGCATGCA 2121
 WTDN15c9          CAGAGAGCCCTTGGGGATACGGATGAAACACACTCTTTCAGCGCGCGTTTACTCGCATGCA 2120
 WTDA21c4          CAGAGAGCCCTTGGGGATACGGATGAAACACACTCTTTCAGCGCGCGTTTACTCGCATGCA 2127
 WTDA21c5          CAGAGAGCCCTTGGGGATACGGATGAAACACACTCTTTCAGCGCGCGTTTACTCGCATGCA 2126
 WTDA21c6          CAGAGAGCCCTTGGGGATACGGATGAAACACACTCTTTCAGCGCGCGTTTACTCGCATGCA 2130
 Cow139c10         CAGAGAGCCCTTGGGGATACGGGTGAAACACACTCTCCGGCACGCGTTTACTCGCATGCA 2132
 Cow139c11         CAGAGAGCCCTTGGGGATACGGATGAAACACACTCTCCGGCACGCGTTTACTCGCATGCA 2133
 Cow2c4            CAGAGAGCCCTTGGGGATACGGATGAAACACACTCTCCGGCACGCGTTTACTCGCATGCA 2133
 Cow133c1          CAGAGAGCCCTTGGGGATACGGATGAAACACACTCTCCGGCACGCGTTTACTCGCATGCA 2132
 Cow139c9          CAGAGAGCCCTTGGGGATACGGATGAAACACACTCTCCGGCACGCGTTTACTCGCATGCA 2132
 Cow3535c3         CAGAGAGCCCTTGGGGATACGGATGAAACACACTCTCCGGCACGCGTTTACTCGCATGCA 2132
 Cow3535c6         CAGAGAGCCCTTGGGGATACGGATGAAACACACTCTCCGGCACGCGTTTACTCGCATGCA 2132
 Cow2c7            CAGAGAGCCCTTGGGGATACGGATGAAACACACTCTCCGGCACGCGTTTACTCGCATGCA 2132
 Cow2c10           CAGAGAGCCCTTGGGGATACGGATGAAACACACTCTCCGGCACGCGTTTACTCGCATGCA 2132
 AB007814Tth       CAGAGAGCCCTTGGGGATACGGATGAAACACACTCTCCGGCACGCGTTTACTCGCATGCA 2132
 Cow104c2          CAGAGAGCCCTTGGGGATACGGATGAAACACACTCTCCGGCACGCGTTTACTCGCATGCA 2133
 Cow104c3          CAGAGAGCCCTTGGGGATACGGATGAAACACACTCTCCGGCACGCGTTTACTCGCATGCA 2133
 Cow104c1          CAGAGAGCCCTTGGGGATACGGATGAAACACACTCTCCGGCACGCGTTTACTCGCATGCA 2133
 Cow2095c4         CAGAGAGCCCTTGGGGATACGGATGAAACACACTCTCCGGCACGCGTTTACTCGCATGCA 2136
 Cow2095c9         CAGAGAGCCCTTGGGGATACGGATGAAACACACTCTCCGGCACGCGTTTACTCGCATGCA 2136
 Cow2095c8         CAGAGAGCCCTTGGGGATACGGATGAAACACACTCTCCGGCACGCGTTTACTCGCATGCA 2136
 WTDA3c9.1         CAGAGAGCCCTTGGGGATACGGATGAAACATA----------------------------- 2082
 WTDA3c9.4         CAGAGAGCCCTTGGGGATACGGATGAAACATA----------------------------- 2082
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**Figure 3** continued

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Elk142c2        GAA-GAGGGAACTGCACTATTTTTT-AGTGTGGTTTTTTTGTGTATGATCCGCTCCGGCG 2186
Elk142c10       GAA-GAGGGAGCTGCACTATTTTTTTAGTGTGGTTTTTTTGTGTATGATCCGCTCCGGCG 2189
Elk142c5        GAA-GAGGGAGCTGCACTATTTTTTTAGTGTGGTTTTTTTGTGTATGATCCGCTCCGGCG 2166
Elk328c3        GAA-GAGGGAACTATACTATTCTTT-AGTGTGGTTTTTTTGTGTATGATCCGCTCCGGCG 2181
Elk416c8        GAA-GAGGGAACTATACTATTCTTT-AGTGTGGTTTTTTTGTGTATGATCCGCTCCGGCG 2181
WTDN15c1        GAA-GATGGAACTATACTATTTTTT-AGTGTGGTTTTTTCGTGTATGATCCGCTCCGGCG 2183
WTDNL15c6       GAA-GATGGAACTATACTATTTTTT-AGTGTGGTTTTTTCGTGTATGATCCGCTCCGGCG 2179
WTDN15c9        GAA-GATGGAACTATACTATTTTTT-AGTGTGGTTTTTTCGTGTATGATCCGCTCCGGCG 2178
WTDA21c4        GAA-GAGGGAACTATACTATTTTTT-AGTGTGGTTTTTTCGTGTATGATCCGCTCCGGCG 2185
WTDA21c5        GAA-GAGGGAACTATACTATTTTTT-AGTGTGGTTTTTTCGTGTATGATCCGCTCCGGCG 2184
WTDA21c6        GAA-GAGGGAACTATACTATTTTTT-AGTGTGGTTTTTTCGTGTATGATCCGCTCCGGCG 2188
Cow139c10       GAAAGAGAGGGCTACACTATTTTTTTAGTGTGGTATTTTTGTGTATGATCCGCTCCGGCG 2192
Cow139c11       GAAAGAGAGGGCTACACTATTTTTTTAGTGTGGTATTTTTGTGTATGATCCGCTCCGGCG 2193
Cow2c4          GAAAGAGAGGGCTACACTATTTTTTTAGTGTGGTATTTTTGTGTATGATCCGCTCCGGCG 2193
Cow133c1        GAAAGAGAGGGCTACACTATTTTTTTAGTGTGGTATTTTTGTGTATGATCCGCTCCGGCG 2192
Cow139c9        GAAAGAGAGGGCTACACTATTTTTTTAGTGTGGTATTTTTGTGTATGATCCGCTCCGGCG 2192
Cow3535c3       GAAAGAGAGGGCTACACTATTTTTTTAGTGTGGTATTTTTGTGTATGATCCGCTCCGGCG 2192
Cow3535c6       GAAAGAGAGGGCTACACTATTTTTTTAGTGTGGTATTTTTGTGTATGATCCGCTCCGGCG 2192
Cow2c7          GAAAGAGAGGGCTACACTATTTTTTTAGTGTGGTATTTTTGTGTATGATCCGCTCCGGCG 2192
Cow2c10         GAAAGAGAGGGCTACACTATTTTTTTAGTGTGGTATTTTTGTGTATGATCCGCTCCGGCG 2192
AB007814Tth     GAAAGAGAGGGCTACACTATTTTTTTAGTGTGGTATTTTTGTGTATGATCCGCTCCGGCG 2192
Cow104c2        GAAAGAGAGGGCTACACTATTTTTTTAGTGTGGTATTTTTGTGTATGATCCGCTCCGGCG 2193
Cow104c3        GAAAGAGAGGGCTACACTATTTTTTTAGTGTGGTATTTTTGTGTATGATCCGCTCCGGCG 2193
Cow104c1        GAAAGAGAGGGCTACACTATTTTTTTAGTGTGGTATTTTTGTGTATGATCCGCTCCGGCG 2193
Cow2095c4       GAAAGAGAGGGCTACACTATTTTTTTAGTGTGGTATTTTTGTGTATGATCCGCTCCGGCG 2196
Cow2095c9       GAAAGAGAGGGCTACACTATTTTTTTAGTGTGGTATTTTTGTGTATGATCCGCTCCGGCG 2196
Cow2095c8       GAAAGAGAGGGCTACACTATTTTTTTAGTGTGGTATTTTTGTGTATGATCCGCTCCGGCG 2196
WTDA3c9.1       ------------------------------------TTTTGTGTATGATCCGCTCCGGCG 2106
WTDA3c9.4       ------------------------------------TTTTGTGTATGATCCGCTCCGGCG 2106
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**Figure 3** continued

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Elk142c2        CTTATGTGTGTGTGCGTGTG--CTCCTTCCTCTCTCACTTTGTGTACTCATGT-----AT 2239
Elk142c10       CTTATGTGTGTGTGCGTGTG--CTCCTTCCTCTCTCACTTTGTGTACTCATGT-----AT 2242
Elk142c5        CTTATGTGTGTGTGCGTGTG--CTCCTTCCTCTCTCACTTTGTGTACTCATGT-----AT 2219
Elk328c3        CTTGTGTGTGTG--CGTGTG--CTCCTTCCTCTCTCACTTTGTGCTCATATGT-----GT 2232
Elk416c8        CTTGTGTGTGTGTGCGTGTG--CTCCTTCCTCTCTCACTTTGTGCTCATATGT-----GT 2234
WTDN15c1        CTTGTGTGTGTGTGCGTGTG--CTCCTTCCTCTCTCACTTTGTGCTCATATGT-----GT 2236
WTDNL15c6       CTTGTGTGTGTGTGCGTGTG--CTCCTTCCTCTCTCACTTTGTGCTCATATGT-----GT 2232
WTDN15c9        CTTGTGTGTGTGTGCGTGTG--CTCCTTCCTCTCTCACTTTGTGCTCATATGT-----GT 2231
WTDA21c4        CTTGTGTGTGTGTGCGTGTG--CTCCTTCCTCTCTCACTTTGTGCTCATATGT-----GT 2238
WTDA21c5        CTTGTGTGTGTGTGCGTGTG--CTCCTTCCTCTCTCACTTTGTGCTCATATAT-----GT 2237
WTDA21c6        CTTGTGTGTGTGTGCGTGTG--CTCCTTCCTCTCTCTCTTTGTGCTCATATAT-----GT 2241
Cow139c10       CTTGTGTGTGTGTGCGTGTA--CTCCTTCCTCTCTCACTTTGTACTCATATGT-----AT 2245
Cow139c11       CTTGTGTGTGTGTGCGTGTG--CTCCTTCCTCTCTCACTTTGTACTCATATGT-----AT 2246
Cow2c4          CTTGTGTGTGTGTGCGTGTG--CTCCTTCCTCTCTCACTTTGTACTCATATGT-----AT 2246
Cow133c1        CTTGTGTGTGTGTGCGTGTG--CTCCTTCCTCTCTCACTTTGTACTCATATGT-----AT 2245
Cow139c9        CTTGTGTGTGTGTGCGTGTG--CTCCTTCCTCTCTCACTTTGTACTCATATGT-----AT 2245
Cow3535c3       CTTGTGTGTGTGTGCGTGTG--CTCCTTCCTCTCTCACTTTGTACTCATATGT-----AT 2245
Cow3535c6       CTTGTGTGTGTGCGCGTGTG--CTCCTTCCTCTCTCACTTTGTACTCATATGT-----AT 2245
Cow2c7          CTTGTGTGTGTGTGCGTGTG--CTCCTTCCTCTCTCACTTTGTACTCACATGT-----AT 2245
Cow2c10         CTTGTGTGTGTGTGCGTGTG--CTCCTTCCTCTCTCACTTTGTACTCATATGT-----AT 2245
AB007814Tth     CTTGTGTGTGTGTGCGTGTG--CTCCTTCCTCTCTCACTTTGTACTCATATGT-----AT 2245
Cow104c2        CTTGTGTGTGTGTGCGTGTG--CTCCTTCCTCTCTCACTTTGTACTCATATGT-----AT 2246
Cow104c3        CTTGTGTGTGTGTGCGTGTG--CTCCTTCCTCTCTCACTTTGTACTCATATGT-----AT 2246
Cow104c1        CTTGTGTGTGTGTGCGTGTG--CTCCTTCCTCTCTCACTTTGTACTCATATGT-----AT 2246
Cow2095c4       CTTGTGTGTGTGTGCGTGTG--CTCCTTCCTCTCTCACTTTATACTCATATGT-----AT 2249
Cow2095c9       CTTGTGTGTGTGTGCGTGTG--CTCCTTCCTCTCTCACTTTATACTCATATGT-----AT 2249
Cow2095c8       CTTGTGTGTGTGTGCGTGTG--CTCCTTCCTCTCTCACTTTGTACTCATATGT-----AT 2249
WTDA3c9.1       CTTGTGTGTGTGTGCGTGTGTTCTCTTTCCTCTCTCACATTTTGCTCGTGTGTGTAAAAT 2166
WTDA3c9.4       CTTGTGTGTGTGTGCGTGTGTTCTCTTTCCTCTCTCACATTTTGCTCGTGTGTGTAAAAT 2166
                *** ********  *****   *** ********** * ** *   *   * *      *
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**Figure 3** continued

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//
Elk142c2         CATGTCTGTACAACAACAACCAAAAACACCAACGAGAGAAAAAGATAA-AAAAGGATTG- 2331
Elk142c10        CATGTCTGTACAACAACAACCAAAAACACCAACGAGAGAAAAAGATAA-AAAAGGATTG- 2334
Elk142c5         CATGTCTGTACAACAACAACCAAAAACACCAACGAGAGAAAAAGATAA-AAAAGGATTG- 2311
Elk328c3         CATGTCTGTACAACAACAACCAAAAACACCAACGAGAGAAAAAGACAA-AAAAGGATTG- 2324
Elk416c8         CATGTCTGTACAACAACAACCAAAAACACCAACGAGAGAAAAAGACAA-AAAAGGATTG- 2326
WTDN15c1         CATGTCTGTACAACAACAACCAAAAACACCAACGAGAGAAAAAGATAA-AAAGGGATTG- 2328
WTDNL15c6        CATGTCTGTACAACAACAACCAAAAACACCAACGAGAGAAAAAGATAA-AAAAGGATTG- 2324
WTDN15c9         CATGTCTGTACAACAACAACCAAAAACACCAACGAGAGAAAAAGATAA-AAAAGGATTG- 2323
WTDA21c4         CATGTCTGTACAACAACAACCAAAAACACCAACGAGAGAAAAAGATAA-AAAAGGATTG- 2330
WTDA21c5         CATGTCTGTACAACAACAACCAAAAACACCAACGAGAGAGAAAGATAA-AAAAGGATTG- 2329
WTDA21c6         CATGTCTGTACAACAACAACCAAAAACACCAACGAGAGAAAAAGATAA-AAAAGGATTG- 2355
Cow139c10        CATGTCTGTACAACAACAACCAAAAACACCAACGAGAGAAAAAGATAAGAAAAGGATTG- 2345
Cow139c11        CATGTCTGTACAACAACAACCAAAAACACCAACGAGAGAAAAAGATAAGAAAAGGATTG- 2347
Cow2c4           CATGTCTGTACAACAACAACCAAAAACACCAACGAGAGAAAAAGATAAGAAAGGGATTG- 2347
Cow133c1         CATGTCTGTACAACAACAACCAAAAACACCAACGAGAGAAAAAGATAAGAAAAGGATTG- 2346
Cow139c9         CATGTCTGTACAACAACAACCAAAAACACCAACGAGAGAAAAAGATAAGAAAAGGATTG- 2346
Cow3535c3        CATGTCTGTACAACAACAACCAAAAACACCAACGAGAGAAAAAGATAAGAAAAGGATTG- 2346
Cow3535c6        CATGTCTGTACAACAACAACCAAAAACACCAACGAGAGAAAAAGATAAGAAAAGGATTG- 2346
Cow2c7           CATGTCTGTACAACAACAACCAAAAACACCAACGAGAGAAAAAGATAAGAAAAGGATTG- 2346
Cow2c10          CATGTCTGTACAACAACAACCAAAAACACCAACGAGAGAAAAAGATAAGAAAAGGATTG- 2346
AB007814Tth      CATGTCTGTACAACAACAACCAAAAACACCAACGAGAGAAAAAGATAAGAAAAGGATTG- 2346
Cow104c2         CATGTCTGTGCAACAACAACCAAAAACACCAACGAGAGAAAAAGATAAGAAAAGGATTG- 2347
Cow104c3         CATGTCTGTGCAACAACAACCAAAAACACCAACGAGAGAAAAAGATAAGAAAAGGATTG- 2347
Cow104c1         CATGTCTGTACAACAACAACCAAAAACACCAACGAGAGAAAAAGATAAGAAAAGGATTG- 2347
Cow2095c4        CATGTCTGTACAACAACAACCAAAAACACCAACGAGAGAAAAAGATAAGAAAAGGATTG- 2350
Cow2095c9        CATGTCTGTACAACAACAACCAAAAACACCAACGAGAGAAAAAGATAAGAAAAGGATTG- 2350
Cow2095c8        CATGTCTGTACAACAACAACCAAAAACACCAACGAGAGAAAAAGATAAGAAAAGGATTG- 2350
WTDA3c9.1        CATGTCTGTACAACAACAACCAAAAACACCAACGAGAGAAAAAGATAA--AAAGGATTGT 2284
WTDA3c9.4        CATGTCTGTACAACAACAACCAAAAACACCAACGAGAGAAAAAGATAA--AAAGGATTGT 2284
                 ********* ***************************** ***** **   ** ******
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**Figure 3** continued

```
Elk142c2           -GGGCTT--CGGTCTCTCTTCTT--TTTTTCTTTTCCTA--CGCCATGTGTGTATGGTAT 2384
Elk142c10          -GGGCTT--CGGTCTCTCTTCTT--TTTTTCTTTTCCTA--CGCCATGTGTGTATGGTAT 2387
Elk142c5           -TGGCTT--CGGTCTCTCTTCTT--TTTTTCTTTTCCTA--CGCCATGTGTGTATGGTAT 2364
Elk328c3           -GGGCTT--AGGTCTCTCTTCTT--TTTTTCTTTTCCTG--CGCCATGTGTGTATGGTAT 2377
Elk416c8           -GGGCTT--AGGTCTCTCTTCTT--TTTTTCTTTTCCTG--CGCCATGTGTGTATGGTAT 2379
WTDN15c1           -GGGCTT--AGGTCTCTCTTCTT--TTTTTCTTTTCCTG--CGCCATGTGTGTATGGTAT 2381
WTDNL15c6          -GGGCTT--AGGTCTCTCTTCTT--TTTTTCTTTTCCTG--CGCCATGTGTGTATGGTAT 2377
WTDN15c9           -GGGCTT--AGGTCTCTCTTCTT--TTTTTCCTTTCCTG--CGCCATGTGTGTATGGTAT 2376
WTDA21c4           -GGGCTT--AGGTCTCTCTTCTT--TTTTTCTTTTCCTG--CGCCATGTGTGTATGGTAT 2383
WTDA21c5           -GGGCTT--AGGTCTCTCTTCTT--TTTTTCTTTTCCTG--CGCCATGTGTGTATGGTAT 2382
WTDA21c6           -GGACTT--AGGTCTCTCTTCTT--TTTTTCTTTTCCTG--CGCCATGTGTGTATGGTAT 2408
Cow139c10          -GGGCTT--AGGTCTCTCTTCTT-TTTTTTCTTTTCCTA--CGCCATGTGTGTATGGTAT 2399
Cow139c11          -GGGCTT--AGGTCTCTCTTCTT-TTTTTTCTTTTCCTA--CGCCATGTGTGTATGGTAT 2401
Cow2c4             -GGGCTT--AGGTCTCTCTTCTT-TTTTTTCTTTTCCTA--CGCCATGTGTGTATGGTAT 2401
Cow133c1           -GGGCTT--AGGTCTCTCTTCTT-TTTTTTCTTTTCCTA--CGCCATGTGTGTATGGTAT 2400
Cow139c9           -GGGCTT--AGGTCTCTCTTCTT-TTTTTTCTTTTCCTA--CGCCATGTGTGTATGGTAT 2400
Cow3535c3          -GGGCTT--AGGTCTCTCTTCTT-TTTTTTCTTTTCCTA--CGCCATGTGTGTATGGTAT 2400
Cow3535c6          -GGGCTT--AGGTCTCTCTTCTT-TTTTTTCTTTTCCTA--CGCCATGTGTGTATGGTAT 2400
Cow2c7             -GGGCTT--AGGTCTCTCTTCTT-TTTTT-CTTTTCCTA--CGCCATGTGTGTATGGTAT 2399
Cow2c10            -GGGCTT--AGGTCTCTCTTCTT-TTTTTTCTTTTCCTA--CGCCATGTGTGTATGGTAT 2400
AB007814Tth        -GGGCTT--AGGTCTCTCTTCTT-TTTTTTCTTTTCCTA--CGCCATGTGTGTATGGTAT 2400
Cow104c2           -GGGCTT--AGGTCTCTCTTCTTCTTTTTTCTTTTCCTA--CGCCATGTGTGTATGGTAT 2402
Cow104c3           -GGGCTT--AGGTCTCTCTTCTTCTTTTTTCTTTTCCTA--CGCCATGTGTGTATGGTAT 2402
Cow104c1           -GGGCTT--AGGTCTCTCTTCTTCTTTTTTCTTTTCCTA--CGCCATGTGTGTATGGTAT 2402
Cow2095c4          -GGGCTT--AGGTCTCTCTTCTT-TTTTTTCTTTTCCTA--CGCCATGTGTGTATGGTAT 2404
Cow2095c9          -GGGCTT--AGGTCTCTCTTCTT-TTTTTTCTTTTCCTA--CGCCATGTGTGTATGGTAT 2404
Cow2095c8          -GGGCTT--AGGTCTCTCTTCTT-TCTTTTCTTTTCCTA--CGCCATGTGTGTATGGTAT 2404
WTDA3c9.1          GGGGCTTTACGGTCTCTCTTTT-CTTTTTTCTTTTCCTGTGCGCCATGTGTGTATGGTAT 2343
WTDA3c9.4          GGGGCTTTACGGTCTCTCTTTT-CTTTTTTCTTTTCCTGTGCGCCATGTGTGTATGGTAT 2343
                     * ***   ********** *    *** * ******    ******************
```

**Figure 3** continued

**Table 8.** *Trypanosoma* rDNA sequences obtained in this study

| Sample | Clone Number | Sequence ID | Gene Region | Amplicon Size | GenBank Accession No. |
|---|---|---|---|---|---|
| Cow 138 | 2 | Cow 138 Cl2 | 18S | 2143 | JX178190 |
| Cow 138 | 4 | Cow 138 Cl4 | 18S | 2143 | JX178191 |
| Cow 2073 | 7 | Cow 2073 Cl7 | 18S | 2143 | JX178181 |
| Cow 2073 | 9 | Cow 2073 Cl9 | 18S | 2117 | JX178182 |
| Cow 2095 | 8 | Cow 2095 Cl8 | ITS | 2212 | JX178162 |
| Cow 2095 | 9 | Cow 2095 Cl9 | ITS | 2212 | JX178163 |
| Cow 2 | 4 | Cow 2 Cl4 | ITS | 2212 | JX178164 |
| Cow 2 | 7 | Cow 2 Cl7 | ITS | 2212 | JX178165 |
| Cow 2 | 10 | Cow 2 Cl10 | ITS | 2212 | JX178166 |
| Cow 3535 | 3 | Cow 3535 Cl3 | ITS | 2212 | JX178167 |
| Cow 3535 | 6 | Cow 3535 Cl6 | ITS | 2212 | JX178168 |
| WTD A1 | 1 | WTD A1 Cl1 | 18S | 2143 | JX178192 |
| WTD A1 | 4 | WTD A1 Cl4 | 18S | 2117 | JX178193 |
| WTD A21 | 4 | WTD A21 Cl4 | 18S | 2049 | JX178194 |
| WTD A21 | 6 | WTD A21 Cl6 | 18S | 2049 | JX178195 |
| WTD A5 | 1 | WTD A5 Cl1 | 18S | 2117 | JX178196 |
| WTD 148 | 4 | WTD 148 Cl4 | 18S | 2117 | JX178197 |
| WTD NL15 | 6 | WTD NL15 Cl6 | ITS | 2211 | JX178170 |
| WTD NL15 | 1 | WTD NL15 Cl1 | ITS | 2211 | JX178169 |
| WTD NL15 | 9 | WTD NL15 Cl9 | ITS | 2211 | JX178171 |
| WTD A3 | 9.1 | WTD A3 Cl9.1 | ITS | 2179 | JX178172 |
| WTD A3 | 9.4 | WTD A3 Cl9.4 | ITS | 2179 | JX178173 |

The majority of differences were found in only two subclones from WTD A3 clone 9 (9.1 and 9.4), with the rest of the sequences being highly similar (Fig. 3; Table 9). At reference *T. theileri* (AB007415Tth) rDNA base pair positions 155, 166, 410, 461, 1736, 1809, 1821, 2234, 2244 and 2335 all of the elk and WTD trypanosome sequences hold the same respective bases which are different than those held by all of the cattle trypanosome sequences and the GenBank *T. theileri* sequence (color indicated in the alignment, Fig. 3). However, at reference *T. theileri* base pair positions 1667 and 1743 the WTD A3 subclones 9.1 and 9.4 trypanosome nucleotides match the cattle clone sequences, including the GenBank *T. theileri* sequence while the rest of the WTD and all of the elk trypanosome sequences match each other. At *T. theileri* base pair position 1662, 1691, 1737, 2109, 2111, 2140, and 2167 the WTD A3 subclones 9.1 and 9.4 nucleotides are different from all of the other sequences. At these positions the rest of the WTD trypanosome sequences match the elk sequences, and the cattle sequences match the GenBank *T. theileri* reference sequence. At the reference *T. theileri* base positions 1751 and 2379, the cattle nucleotides match the reference sequence, two of the elk (328 and 416) trypanosome sequences match the cattle parasite and three match the WTD parasite sequences, all of which are the same. At position 2172, the elk, WTDA3 subclones 9.1 and 9.4, and cattle trypanosome nucleotides are all the same, but the other WTD match each other. At positions 2143 and 2147, the elk trypanosome sequences match either the WTD or the cattle parasite sequences, and the WTDA3 subclones 9.1 and 9.4 have deletions. The trypanosome sequences from WTDA3 subclones 9.1 and 9.4 are notably distinct in sequence compared to the other isolates, as shown in Figure 2 (noted in green font).

**Table 9.** Notable nucleotide substitutions in trypanosome ribosomal DNA

| No.* | 1 | 2 | 3 | 4 | 5 | 6 | 7 | 8 |
|---|---|---|---|---|---|---|---|---|
| BP position | **155** | **166** | **410** | **461** | **1662** | **1667** | **1691** | **1736** |
| Elk | **C** | **G** | **G** | **C** | **T** | **A** | **A** | **A** |
| WTD | **C** | **G** | **G** | **C** | **T** | **A** | **A** | **A** |
| Cattle | **A** | **A** | **A** | **T** | **C** | **G** | **G** | **C** |
| WTD 3 | **C** | **G** | **G** | **C** | **G** | **G** | **del**** | **A** |
| No. | 9 | 10 | 11 | 12 | 13 | 14 | 15 | 16 |
| BP position | **1737** | **1743** | **1751** | **1809** | **1821** | **2109** | **2111** | **2140** |
| Elk | **A** | **A** | **T/G** | **T** | **del** | **T** | **A** | **G** |
| WTD | **A** | **A** | **G** | **T** | **del** | **T** | **A** | **G** |
| Cattle | **T** | **G** | **T** | **A** | **G** | **C** | **G** | **A** |
| WTD 3 | **del** | **G** | **G** | **T** | **del** | **del** | **del** | **del** |
| No. | 17 | 18 | 19 | 20 | 21 | 22 | 23 | 24 |
| BP position | **2143** | **2147** | **2167** | **2172** | **2234** | **2244** | **2335** | **2379** |
| Elk | **R** | **Y** | **T** | **T** | **G** | **A** | **del** | **R** |
| WTD | **A** | **T** | **T** | **C** | **G** | **G** | **del** | **G** |
| Cattle | **G** | **C** | **A** | **T** | **A** | **A** | **G** | **A** |
| WTD 3 | **del** | **del** | **del** | **T** | **G** | **A** | **del** | **G** |

*Number indicated in alignment, Figure 3
**del, deletion

Overall, the phylogenetic analyses show the cattle *Trypanosome* 18S rDNA sequences, both those from the U.S. (the current study) and Japan (GenBank Accession Nos. AJ009163 and AJ009164), diverging more recently from the other *Trypanosoma* spp. (Figs. 4-9).

The phylogenetic tree of the trypanosome 18S region of approximately 1200 bp derived from the 18S amplicon sequences shows a major clade holding the WTD sequences interspersed among cattle and elk sequences (Fig. 4) with strong bootstrap support (100). Within the major clade, MN Elk 317 clones 3 and 4 split from the other sequences, forming a separate well supported (100) branch. The other branch, holding the remaining elk and the WTD and cattle sequences, is strongly supported (82). This latter branch then splits into a moderately supported cluster (52) holding WTD A5 and A1 clone 1 on one branch, and Cow 138 clone 2, WTD A21 Clone 4, Cow 138 cl 4, WTD A1 clone 4 and WTD A21 clone 5 on another. Included in the clade, but branching separately, are MN elk 328 clone 21, WTD 148 clone 4, WTD A1 clone 4 and WTD A21 clone 4. The second cluster consists of the cattle *Trypanosoma* sequences from Cow 2073 (clones 7 and 9), *T. theileri* from Japan and, branching separately, *Trypanosoma* sp. from a WTD, *Cervus dama* (Fig. 5). Within the clade, but branching separately are WTD A21 clone 6 and MN Elk 328 clone 20.

Ancestral to this major clade, but still within the grouping, are sequences from *T. rangeli* and *T. leuwenhoeki*, which form a branch, and *T. cruzi*, forming its own branch. *T. evansi* and *T. equiperdum* sequences form a separate and divergent clade.

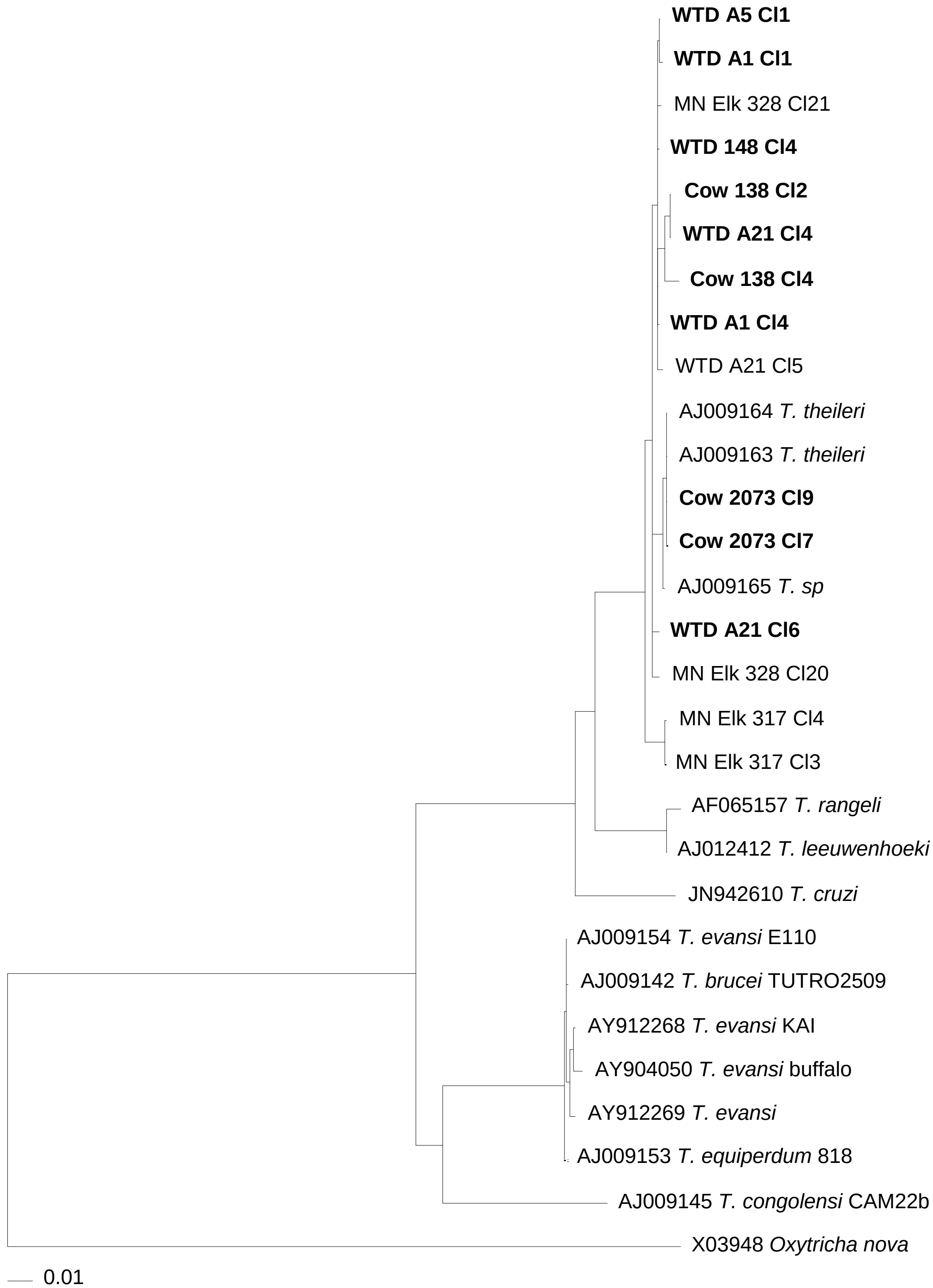


**Figure 4.** Neighbor-joining phylogenetic tree of relationships among cloned *Trypanosoma* sequences obtained from 18S ribosomal DNA PCR. Sequences obtained in this study are in bold type. The *Oxytricha nova* 18S rDNA sequence (Genbank Accession No. X03948) served as the outgroup.

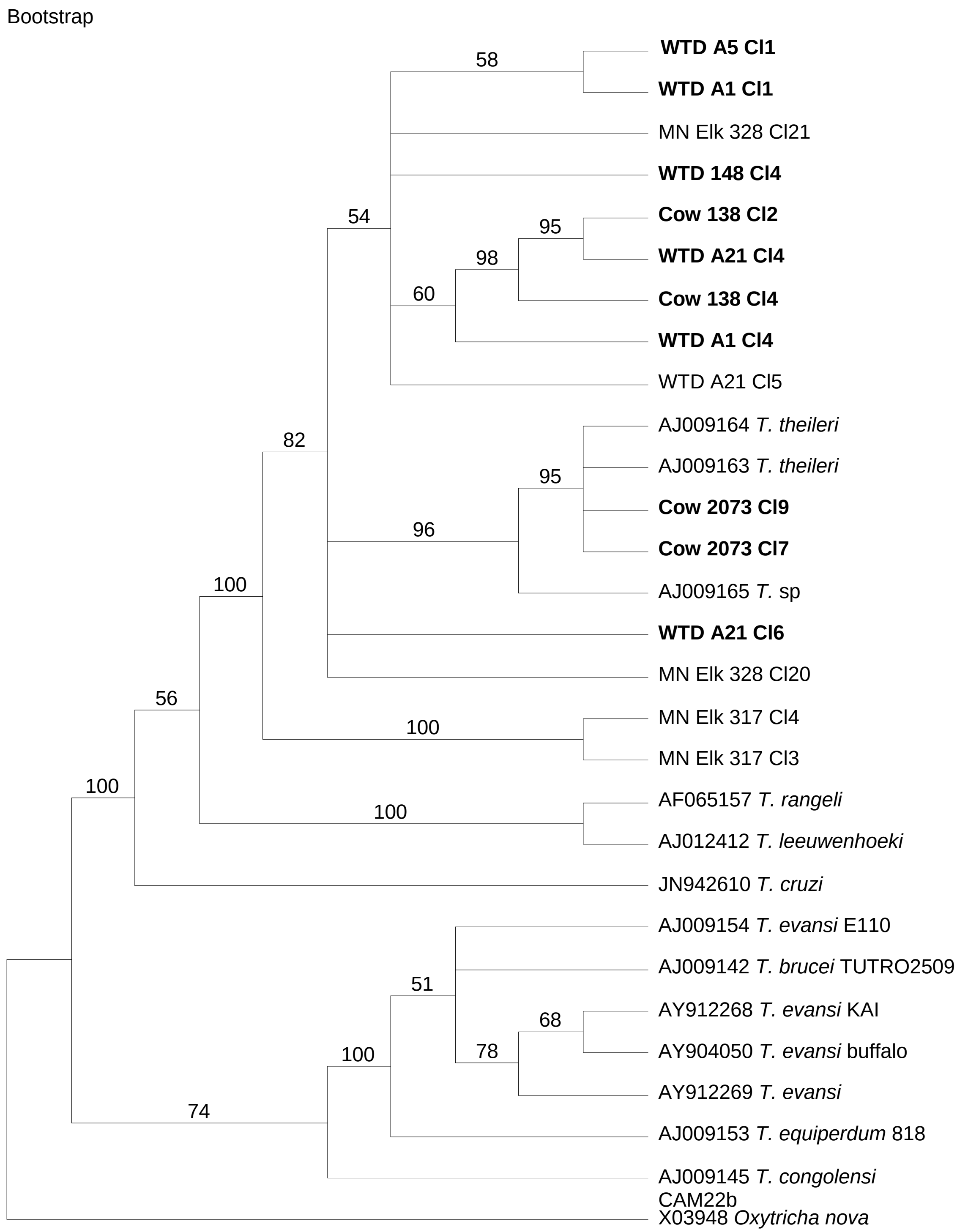


**Figure 5.** Cladogram of relationships among cloned *Trypanosoma* sequences obtained from 18S ribosomal DNA PCR showing bootstrap support (1000 repetitions). Sequences obtained in this study are in bold type. *Oxytricha nova* 18S rDNA sequence (Genbank Accession No. X03948) served as the outgroup.

The phylogenetic tree of the 5' 1500 bp 18S gene region shows most of the cattle *Trypanosoma* sequences clustering together with the *T. theileri* sequence from cattle obtained from the GenBank database (Accession No. AB007814) (Fig. 6). The *T. evansi* isolates (GenBank Accession Nos. AB551921, AJ009142, AJ009154, AY912269, AY904050, and AY912268) form a clade on their own branch with bootstrap analysis showing strong support (100) (Figs. 6 and 7). Next, the GenBank sequences from *T. rangeili* and *T. leeuwenhoeki* (GenBank Accession Nos. AF065157 and AJ012412) are shown to be ancestral to the rest of the sequences (Fig. 6) with 100 bootstrap support (Fig. 7). The *Trypanosoma* sequences from WTD A3 subclones 9.1 and 9.4 along with cattle *T. theileri* isolates (GenBank Accession Numbers AB569250 and AB569249) form their own cluster with moderate support (74). The WTD, elk, and cattle *Trypanosoma* sequences form a major clade with moderate support of 82 that includes a Sika WTD *Trypanosoma* sp. sequence. Within this clade, MN Elk 142 clone 5 and WTD NL15 clone 6 form a single clade with support of 64. The cattle *Trypanosome* sequences from this study and cattle *T. theileri* (AB700814) form a more recently diverged well supported cluster (97) separate from the elk and WTD trypanosome sequences from this study.

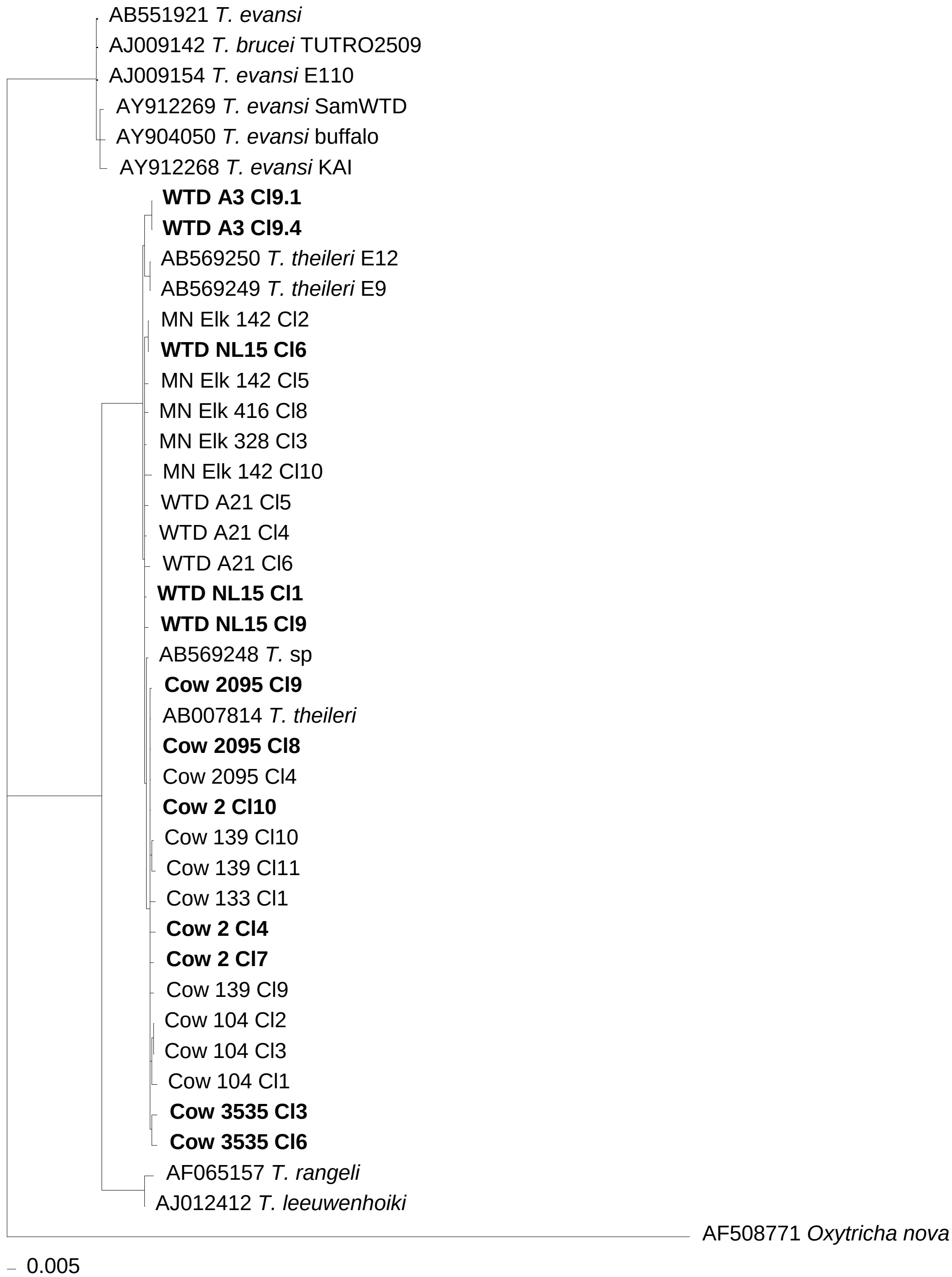


**Figure 6.** Phylogenetic tree of 5' 1500 base pair 18S rRNA gene region. Neighbor-joining phylogenetic tree of relationships among cloned *Trypanosoma* sequences obtained from 18S ribosomal DNA PCR. Sequences obtained in this study are in bold type. The *Oxytricha nova* 18S rDNA sequence (Genbank Accession No. X03948) served as the outgroup.

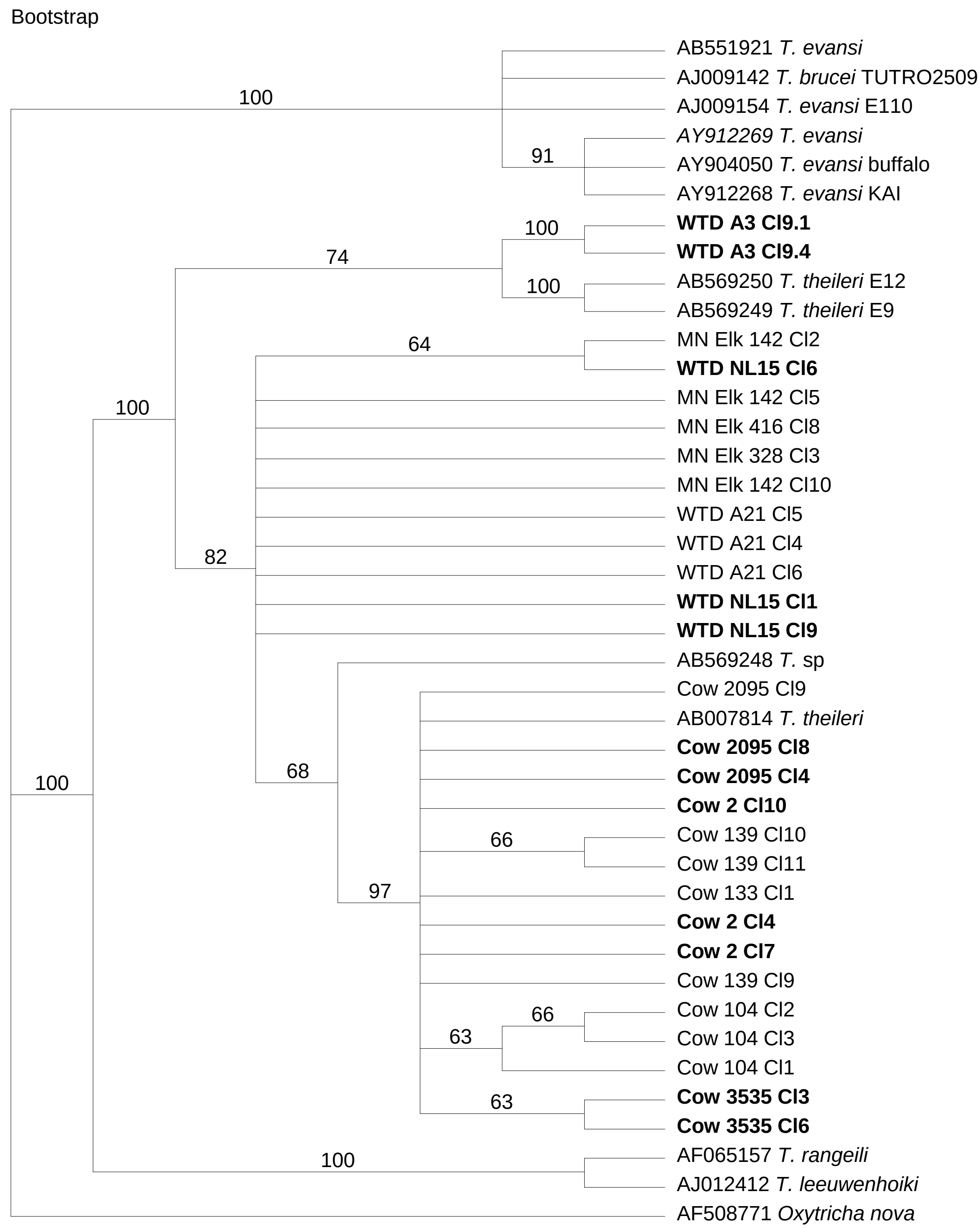


**Figure 7.** Bootstrap of 5' 1500 base pair 18S rRNA gene region. Neighbor-joining phylogenetic tree of relationships among cloned *Trypanosoma* sequences obtained from 18S ribosomal DNA PCR. Sequences obtained in this study are in bold type. The *Oxytricha nova* 18S rDNA sequence (Genbank Accession No. X03948) served as the outgroup.

The phylogenetic tree of the *Trypanosoma* ITS1-5.8S-ITS2 region (Fig. 8) shows a similar topology to the 5' 1500 bp 18S rDNA phylogenetic tree, branching into two clades with one holding WTD sequences A3, subclones 9.1 and 9.4 and cattle *T. theileri* (AB569250 and AB569249) in a distinct clade with 100 support (Fig. 9). The second holds elk, WTD, cattle and Sika deer sequences (85 support). Within this clade, the cattle *Trypanosoma* sequences from this study cluster together with *T. theileri* sequence from cattle (GenBank Accession No. AB007814) with 100 support. Most of the WTD *Trypanosoma* sequences branch with the elk sequences off the same main branch. Three of the elk trypanosome sequences (MN elk 142 clone 2, MN elk 142 clone 10, and MN elk 142 clone 5) form a distinct clade with good support (100). The rest of the elk and WTD sequences form a separate clade with good support (99). Within this clade, MN elk 328 clone 3 and MN elk 416 clone 8 form a small clade with 94 support and branch off before the WTD sequences.

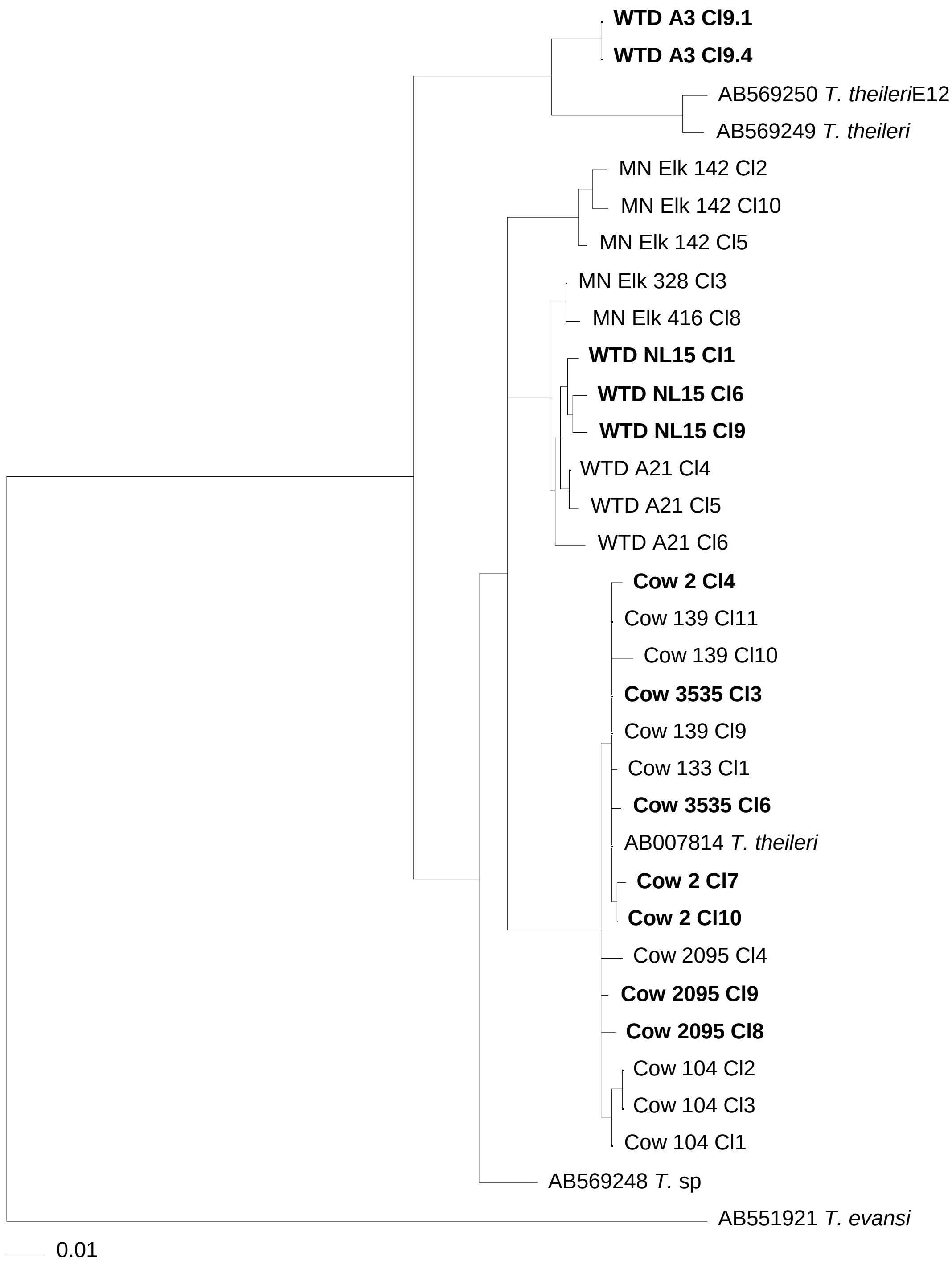


**Figure 8.** Phylogenetic tree of ITS1-5.8S-ITS2 rRNA genomic region. Neighbor-joining phylogenetic tree of relationships among cloned *Trypanosoma* sequences obtained from rRNA PCR. Sequences obtained in this study are in bold type. The *Trypanosoma evansi* ITS1-5.8S-ITS2 rRNA sequence (Genbank Accession No. AB551921) served as the outgroup.

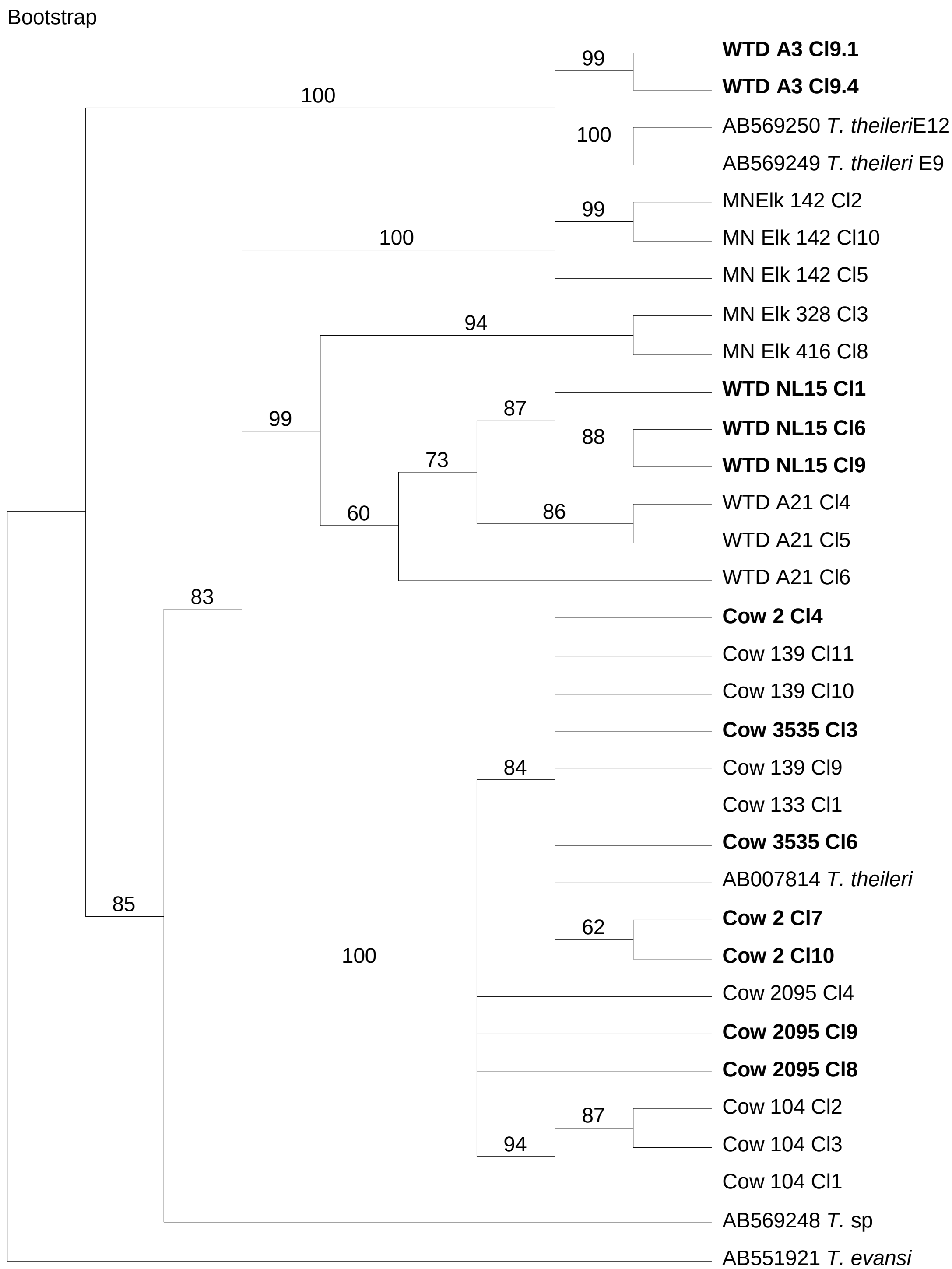


**Figure 9.** Bootstrap of ITS1-5.8S-ITS2 rRNA genomic region. Neighbor-joining phylogenetic tree of relationships among cloned *Trypanosoma* sequences obtained from rRNA PCR. Sequences obtained in this study are in bold type. The *Trypanosoma evansi* ITS1-5.8S-ITS2 rRNA sequence (Genbank Accession No. AB551921) served as the outgroup.

**DISCUSSION**

In the past, *Trypanosoma* parasites from cattle, elk, and WTD have generally only been diagnosed by visualization methods rather than genetic methods. Garcia et al (2011) looked at genetic diversity in trypanosomes of cattle, water buffalo, sheep and WTD in Brazil and Venezuela (Garcia et al., 2011). Cattle, water buffalo, sheep, antelope and WTD trypanosomes were found to be distinct in 18S rRNA, ITS rDNA, 5S rRNA, glycosomal glyceraldehyde 3-phosphate dehydrogenase, mitochondrial cytochrome b, spliced leader, and cathespsin L-like genes and corresponded to separate host species. Cattle and water buffalo were found to be more closely related to each other than to the WTD and antelope *Trypanosoma* isolates.

Elk (*Cervus elaphus canadensis*) and mule deer (*Odocoileus hemionus*) were the first wild ungulates to have the trypanosome parasite morphologically described as *T. cervi* found in their blood stream (Kingston and Morton, 1975a). In the current study, trypanosomes from WTD, presumably *T. cervi,* and cattle trypanosomes, presumably *T. theileri,* were compared molecularly using ribosomal DNA sequence comparisons. Because the original *T. cervi* was described in elk, isolates from this host were also compared to the trypanosome sequences obtained in this study.

Two phylogenetic trees were constructed of 18S rRNA gene region sequences, one of a 1200 bp region at the beginning (3' end) of the 18S rRNA gene and one of the last 1500 bp of the gene (5' end). The *Trypanosoma* sequences used in each tree were obtained from different groups of animals. The tree that spanned the more 3' portion of the 18S rRNA gene region (Fig. 4) showed the cattle, WTD, and elk sequences intermingled, whereas the tree for the region spanning 1500 bp from the 5' end clearly separated the WTD and cattle trypanosome sequences from each other (Fig. 6). Although this disparity might be explained by the fact that the 3' end sequences may be more highly conserved across *Trypanosoma* species compared to the 5' end, a review of the aligned sequences reveals that two sites that clearly separate the cattle from the WTD trypanosome sequences in the 1500 bp 18S rDNA fragment (positions 145 and 156 in Fig. 4) are not informative in the 1200 bp fragment (data not shown). Instead, it appears that the trypanosomes represented in the 1200 bp 18S rDNA tree are not host-specific. This is supported by a summary of the informative sites that shows clear overlap between trypanosome sequences from the different hosts (Fig. 3).

The bootstrap analysis of the two 18S regions sequenced found less support between the isolates, where bootstrap analysis strongly supported the clustering in the ITS1-5.8S-ITS2 analysis between the different tree branches. The ClustalW found no differences between sequences in the 5.8S region. The ITS1 and ITS2 regions may be better for future species variation diagnosis than the 18S region and the 5.8S region.

Sequence analysis of an alignment of the genomic region spanning the 5' end of the 18S rRNA gene through the ITS1, 5.8S rRNA gene, and ITS2 revealed 24 nucleotide positions that appeared to be specific differences between cattle and wild ungulate trypanosomes. The division between cattle sequences and the WTD plus elk sequences was not complete; four of the positions had WTD A3 subclones 9.1 and 9.4 nucleotides aligning with those found in all of the cattle trypanosome sequences. Four sequence positions had elk trypanosome nucleotides aligning with those of cattle trypanosomes. It is possible that these elk and WTD trypanosome sequences are in fact the same *Trypanosoma* species found in cattle; ergo, elk and WTD are able to harbor cattle species. The phylogenetic trees for the 18S rRNA gene region amplified along with the ITS1-5.8S-ITS2 region (5' 1500 bp 18S rDNA) and that for the ITS1-5.8S-ITS2 region itself (Figs. 6 and 8) showed most of the WTD and elk trypanosome sequences clustering while cattle sequences clustered with isolates obtained from cattle, with a few exceptions. Two of the trypanosome sequences from WTD aligned with cattle better than WTD and elk sequences at certain base pair orientations, which could indicate that these sequences are alleles or the same species found in cattle.

The phylogenetic tree of the 18S rRNA gene region derived from fragments of approximately 1200 base pairs shows an amalgamating mixture of WTD, elk and cattle trypanosome sequences. Although the major clade holds some of the cattle parasite sequences aligned with those from WTD and elk, one internal node branches into a cluster holding only cattle trypanosome sequences from this study along with *T. theileri* sequence from the GenBank database (Accession No AB007814). This indicates that these particular isolates sequenced from cattle in this study are most likely *T. theileri*. It is possible that the group of cattle, WTD, and elk sequences represent alleles of the same *Trypanosoma* species. It is also possible that the sequences of the 18S region of different *Trypanosoma* spp. are so similar that the phylogenetic tree was unable to differentiate them. Yet another possibility is that cattle can harbor elk and WTD *Trypanosoma* sp. and vice versa. These trees (Figs. 6 and 8) also showed the WTD and elk sequences branching before the cattle sequences, which could mean that the *Trypanosoma* from cattle are diverging from the WTD and elk trypanosomes. Given the short distances, and given how closely *T. evansi* and *T. brucei* align, this may be an ongoing event.

The trypanosome sequences from WTD A3 subclones 9.1 and 9.4 aligned with two cattle *T. theileri* sequences from the GenBank database and diverge from the rest of the WTD, elk, and cattle samples. Since the subclones branched with two cattle *Trypanosomes* it is possible that these are very closely related parasite species and may cross between WTD and cattle. The three cattle *T. theileri* sequences from the GenBank databank branched separately on the phylogenetic tree. As mentioned, two of these isolates (Accession Nos. AB569250 and AB569249) branched with WTD A3 sequences, while the third, GenBank AB007814, branched with the cattle sequences obtained in this study. It is possible that these represent multiple species of *Trypanosoma* in cattle. Alternatively, it is also possible that the two distinct *T. theileri*-type sequences represent alleles of the same species. Arguing against this is the fact that similar separation among the various clones of the trypanosome isolates was not observed.

This study found very close phylogenetic relationships between the WTD and elk trypanosome ribosomal DNA sequences. The phylogenies based on the ITS1-5.8S-ITS2 genomic region and the longer 18S rDNA sequence clearly separated the cattle parasites from the WTD and elk, with the exception of the WTD A3 trypanosomes. Thus, this study suggests that the cattle parasite sequences in this study are from a different species of *Trypanosoma* from those in WTD and elk, and that the cattle sequences represent *T. theileri* and the WTD and elk sequences represent *T. cervi*. It is also possible, however, that the same species infects cattle, elk, and WTD, and that different allele sequences were obtained in this study.

The elk sequences tended to aligned most often with the WTD sequences, but at times the elk aligned with cattle at base pair orientations. Two of the WTD subclone sequences from sample A3 at times aligned with cattle sequences. This could mean that there are additional *Trypanosoma* species that can infect cattle and wild ungulates, or that the different parasite species can cross from wild ungulates to cattle and vice versa. Overall, the cattle sequences aligned with cattle isolates and the WTD sequences with elk sequences, indicating that wild ungulates (WTD and elk) and cattle most likely have separate trypanosome species.

# CHAPTER IV

# CULTURING OF *TRYPANOSOMA* SPP. FROM WTD AND COMPARING MORPHOLOGY TO PREVIOUSLY DESCRIBED SPECIES

## INTRODUCTION

Before Kingston and Morton's reclassification in 1975, trypanosomes of wild ungulates were classified as *Trypanosoma theileri*. *T. theileri* occurs most commonly in the blood of cattle (Levine, 1985). McHolland-Raymond et al. (1978) were able to culture *T.theileri* using Medium 199 with fetal bovine serum, antibiotic-antimycotic, vitamin B12, and Bacto-peptone. The trypomastigote parasite has a long, pointed posterior end with a medium-sized kinetoplast anterior to it and a prominent undulating membrane and a free flagellum.

In 1975, Kingston and Morton classified trypomastigotes from elk and mule WTD as *Trypanosoma cervi* based on morphological and biological distinctions (Matthews et al., 1977). *T. cervi* trypomastigote was found to be a long trypanosome with a short free flagellum and an undulating membrane arising near the kinetoplast (Kingston and Morton, 1975b). The nucleus is large and granular located posteriorly. The kinetoplast is large and usually marginal and posterior to the nucleus. The parasite was cultured using NNN agar slants with trypanosome-free bovine blood as a substitute for rabbit blood (Kingston and Morton, 1975b).

The purpose of this study was to morphologically describe the *Trypanosoma* species found in WTD in Southern Texas using culture.

## MATERIALS AND METHODS

### Parasite Culture

WTD blood samples were provided as collaborative agreements as described in Chapter II. Blood samples were cultured based on packed cell volume, Giemsa stained blood smears, and 18S rRNA results. If blood samples had a low packed cell volume or anything appearing to be a hemoparasite on the original Giemsa stained thin blood smear, they were cultured. Also, if samples were positive on 18S rRNA PCR for *Babesia,* they were cultured. *Trypanosoma* in WTD blood samples were cultured as described in Chapter II with modification. Cultures were initiated by placing 0.25 ml washed red blood cells into one well of a 24- well plate containing 0.75 ml complete culture medium. All samples were cultured in three media. The complete culture medium consisted of either 20% normal adult bovine serum, fetal bovine serum (FBS), or WTD serum supplemented HL-1 medium (Biowhittaker) with l- glutamine and antibiotic-antimycotic (Holman et al., 1993; Holman et al., 1988). Each of the samples was plated in duplicate wells on the plate. The plate was contained in a humidified microisolator containing a 5% carbon dioxide, 2% oxygen and 93% nitrogen atmosphere, and placed in an incubator held at 37 °C.

Culture medium was changed daily until presence of *Trypanasoma* spp was verified, and then the medium was changed every 3 days. To change the medium, 0.8 ml of medium from above the erythrocyte layer was removed and a thin erythrocyte smear was made. Fresh medium (0.8 ml) was then added. Microscopic evaluations of Giemsa-stained thin blood smears were performed at each medium change (Holman, 1988, Holman 1993).

**Image Acquisition**

*Trypanosoma* images were acquired on a light microscope under oil immersion using an optical path mounted camera (Nikon DS-Fi1, Melville, NY) and imaging software (NIS-Elements D 3.1, Melville, NY) was used to prepare images. The images of the *Trypanosoma* spp. were compared to both McHolland-Raymond et al (1978) and Kingston and Morton (1975) for morphological diagnosis.

**RESULTS**

The cultures were checked daily for parasite growth after initiation. Three days after the initiation of the cultures, *Trypanosoma* sp. began to be visualized for all the samples in the wells with FBS medium. At this point, the wells with *Trypanosoma* sp. had less frequent media changes in order to avoid removing *Trypanosoma* parasites. After culturing for an average of 23 days (Table 10), the *Trypanosoma* parasites began to look degraded on thin smear examination. The remaining cultures containing healthy parasites were harvested and banked frozen at -80 °C.

**Table 10.** *Trypanosoma* culture results

| Sample | Serum | Growth of *Trypanosoma* | Length of growth |
|---|---|---|---|
| A3 | FBS | pos | 22 days |
| A3 | WTD | 0 | na |
| A3 | COW | 0 | na |
| A6 | FBS | pos | 21 days |
| A21 | FBS | 0 | na |
| A21 | WTD | 0 | na |
| A21 | COW | 0 | na |
| A22 | FBS | pos | 22 days |
| A22 | WTD | 0 | na |
| A22 | COW | 0 | na |
| A28 | FBS | pos | 21 days |
| A33 | FBS | pos | 30 days |
| A33 | WTD | 0 | na |
| A33 | COW | 0 | na |

Images of the *Trypanosoma* taken from the daily thin blood smears of the cultures were compared with images of cattle *T. theileri.* The image of the cultured *Trypanosoma* (Fig. 10A) showed long trypomastigotes with a thin, pointed posterior end. The kinetoplast was relatively close to the nucleus, but just posterior to it. The undulating membrane was pronounced and there was a free flagellum detached from the anterior end. Dividing forms were present in the culture (Fig. 10B) which indicates there was parasite replication occurring. There also appeared to be a few epimastigotes present in the culture as well. The epimastigote has the kinetoplast posterior to the nucleus and the undulating membrane is less pronounced. The epimastigote free flagellum is shorter and less pronounced than in the trypomastigote. The cattle *T. theileri* (Fig. 11) showed a similar long trypomastigote with a pointed posterior end. The kinetoplast was not as close to the nucleus as observed for the WTD parasite, and the undulating membrane was not as long and was not free from the anterior end.

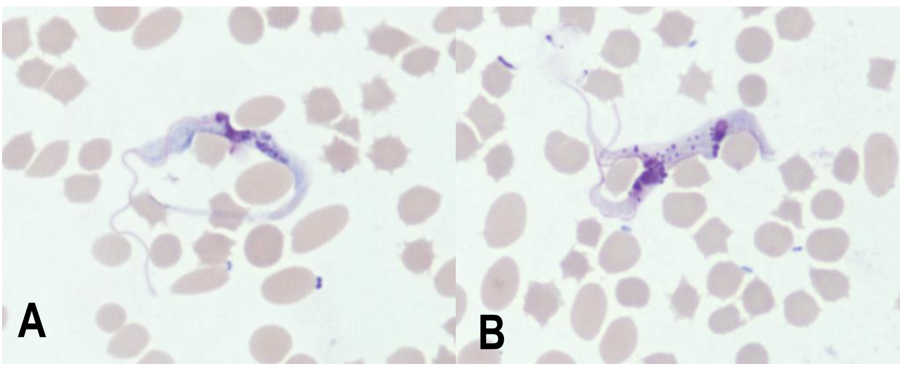


**Figure 10.** *Trypanosoma* forms in WTD erythrocyte culture. A, Trypomastigote; B, dividing form. Giemsa-stained smear at 1000X.

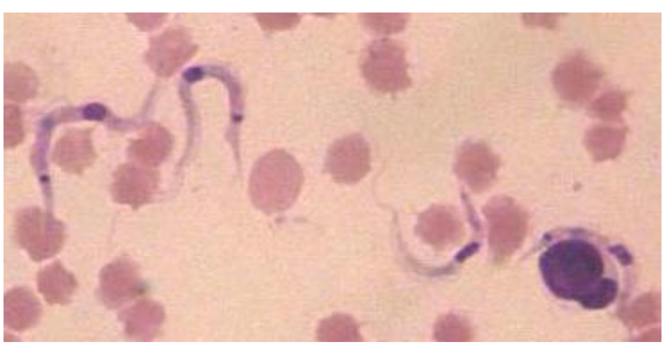

*Photo courtesy of Dr. Thomas Craig,*

*Texas A&M University*

**Figure 11.** *Trypanosoma theileri* trypomastigote from cattle

**DISCUSSION**

The image of the *Trypanosoma* sp. trypomastigote cultured from WTD blood in the current study was similar to images of cattle derived *T. theileri* in that both were long trypomastigotes with a pointed posterior end. *T. theileri* had a kinetoplast that was further away from the nucleus than in the *Trypanosoma* trypomastigote imaged from the WTD culture (Figs. 10 and 11). The *Trypanosoma* cultured in the current study best resembles *T.cervi* with a large nucleus, free flagellum, pronounced undulating membrane, and kinetoplast located posterior to the nucleus as described by Kingston and Morton (1975b). Morphologically, the parasite cultured from WTD in this study appears to be *T. cervi*.

In the past, *T. cervi* was cultured in different tissue media than was used in this study. Kingston and Morton (1975b) were able to culture *T. cervi* epimastigote stage using NNN agar slants with trypanosome-free bovine blood incubated at 22 to 25 °C. In order to study the trypomastigote-like stages, they incubated positive NNN agar slant cultures at 37 °C and also inoculated chick embryos.

McHolland-Raymond et al. (1978) used a similar culture technique to the current study to grow *T. theileri*, using Medium 199 with fetal bovine serum, antibiotic-antimycotic, vitamin B12 (which seemed to be important in the stimulation of trypanosome division), and Bacto-peptone at a pH of 6-7 (McHolland-Raymond et al., 1978). Growth was also investigated in fetal bovine-4-bone marrow cells, adult bovine bone marrow cells, and bovine lymph node cells in the same medium and supported parasite growth.

The current study used normal adult bovine serum, FBS, or WTD serum in HL-1 medium supplemented with l-glutamine and antibiotic-antimycotic. The parasites grew better in the medium that had FBS as the serum source than with the normal adult bovine or the WTD serum. As WTD isolates, it would be expected that the WTD serum would support propagation of the parasite *in vitro*. However, since FBS contains fewer antibodies and more growth factors than adult serum, this could be why the *Trypanosoma* grew best in FBS. Since the parasites were obtained from the blood of WTD and presumably are adapted to this host, this could be why the adult cattle serum did not sustain growth. The adult cattle and adult WTD also may have antibodies to *Trypanosoma* spp. which could have impaired growth in culture.

The growth of the parasites in FBS supplemented medium was continuous for 25 days. In previous studies, continuous growth lasted up to 21 days for agar slants of *T. cervi*. In contrast to the present study, previous studies found a high incidence of epimastigotes in the cultures. Epimastigotes are the *Trypanosoma* life stage form found in insect vectors (Hoare, 1972). There did appear to be potential epimastigotes, as well as dividing epimastigotes in the culture of the present study, but trypomastigotes were more common. This could be due to the presence of mammalian red blood cells and serum in the culture, as well as incubation at 37 °C, leading the parasites to treat the environment as a mammalian host rather than an arthropod host. The basal medium used in this study, HL-1, was developed for cultivation of hybridoma cells, ie. lymphocyte-derived cell lines fused with spleen cells (McVicar et al., 1991). Thus, the formulation of the medium used may have also contributed to the predominance of the trypomastigote stage in the cultures. The morphological difference between the epimastigote and trypomastigote is the location of the kinetoplast relative to the nucleus.

In summary, FBS supplementation of a basal medium designed for blood cell culture supported the in vitro propagation of the *Trypanosoma* sp from WTD in this study. The morphological characteristics of the cultured parasite are consistent with the description for *T. cervi*.

# CHAPTER V

# DISTRIBUTIONAL COMPARISON OF *BABESIA BOVIS* AND *TRYPANOSOMA* SPP. FROM DIFFERENT MAMMALIAN HOSTS WITHIN TEXAS

## INTRODUCTION

*Babesia bovis* has a two host life cycle with three phases including merogony (asexual reproduction) in the vertebrate host, gamogony in the gut of the tick host, and syngamy (asexual reproduction or sporogony) in the cattle fever tick (*Rhipicephalus (Boophilus) annulatus* and *Rhipicephalus (Boophilus) microplus)* salivary glands. The arthropod host injects the infective stage, sporozoites into the mammalian host (Sauer et al., 1995).

*Babesia bovis* was first described by Babes (1888) in Romania associated with red water fever in cattle (Uilenberg, 2006). Severe babesiosis generally occurs in cattle over 3-9 months of age and animals infected as calves have been found to have a higher resistance to infection as adults (Graham and Hourrigan, 1977; Trueman and Blight, 1978).

*Babesia bovis* is an important parasite in cattle production in many areas of the world. In the United States, *B. bovis* was eradicated in 1943 with a program that eradicated the vector, the cattle fever tick (Graham and Hourrigan, 1977; Howell et al., 2007). A buffer zone of quarantined area, 800 km long and 0.4 to 16 km wide, is still present in Texas along the Rio Grande from Brownsville to Del Rio (Graham and

Hourrigan, 1977), but *R. microplus* is encountered on stray Mexican cattle found in Starr, Hidalgo, and Cameron counties in South Texas. Large portions of South Texas have habitat suitable for fever ticks (Pound et al., 2010) and the tick may be introduced via infested livestock and wild ungulates that enter from Mexico where tick eradication efforts have been unsuccessful (George et al., 2002). *Babesia bovis* has not been eradicated in Mexico, which means there is potential for tick infested diseased animals to cross from Mexico into the U.S. If WTD are able to perpetuate cattle *B. bovis,* they would be good reservoirs to spread disease.

Trypanosomes are two-host parasites, consisting of a vertebrate animal and an invertebrate. The invertebrate introduces the metatrypanosome to the mammal by inoculation during a blood meal (Hoare, 1972). *Trypanosoma cervi* has been documented by morphological examination of thin blood smears from numerous ungulate hosts in Europe and the United States. Within the United States, *T. cervi* has been recognized in wild ungulates including WTD in Wyoming, Florida North Carolina, Georgia, Arkansas, Alabama and Alaska (Kingston et al., 1985; Kingston et al., 1982). *Trypanosoma theileri* has been documented most often from cattle (Levine, 1985).

Davies and Clark suggested that the vector for *T. cervi* was the horse fly, a tabanid (Krinsky, 1975). More recently, the ked *Lipoptena mazamae* has been implicated as the vector for *T. cervi* (Samuel et al., 2001). *L. mazamae* is a common blood feeding insect found generally in the southern United States and Central and South America. It has been found on WTD, antelope, and other exotic WTD as well as cattle (Samuel et al., 2001).

The current study compared the occurrence of *Trypanosoma* and *Babesia* found in both cattle and WTD to the region and time of year when the parasite was found.

## MATERIALS AND METHODS

WTD and cattle extracted DNA was provided from previous projects (Chapter II). All samples were processed and extracted as previously described (Chapter II). *Babesia bovis*- and *Trypanosoma*- specific polymerase chain reaction (PCR) was performed on the extracted DNAtargeting the 18S rRNA gene as reported in Chapters II and III. The PCR data was used for comparison between *Babesia* spp. and *Trypanosoma* spp. found in cattle and WTD hosts in different Texas counties and during different seasons in this Chapter. Since the data set was not evenly distributed between counties or seasons, the nonparametric Chi squared test ($\chi^2$) was employed (JMP® 10.0.0, SAS Cary, NC).

## RESULTS

*Trypanosoma* were identified in WTD in three Texas counties, Zapata, Webb, and Starr (Table 11). Within these counties, Starr had the highest occurrence of *Trypanosoma* in WTD with 86.4% (70/81) showing positive bands on 18S rDNA PCR. Starr County also had the lowest occurrence of *Babesia* with 0% (0/81) positive by 18S rDNA PCR.

**Table 11.** WTD PCR results for *Trypanosoma* sp. and *Babesia* sp. in each County

| Date (Month/Year) | Species | Source | Animal ID | County | *Trypanosoma* sp | *Babesia* sp |
|---|---|---|---|---|---|---|
| *WTD in Webb County* | | | | | | |
| Jan-10 | WTD | COOPER | W2 | Webb | POS | NEG |
| Jan-10 | WTD | COOPER | W3 | Webb | POS | NEG |
| Jan-10 | WTD | COOPER | W4 | Webb | NEG | NEG |
| Jan-10 | WTD | COOPER | W5 | Webb | NEG | NEG |
| Jan-10 | WTD | COOPER | W6 | Webb | NEG | NEG |
| Jan-10 | WTD | COOPER | W9 | Webb | NEG | NEG |
| Jan-10 | WTD | COOPER | W12 | Webb | NEG | NEG |
| Jan-10 | WTD | COOPER | W13 | Webb | NEG | NEG |
| Jan-10 | WTD | COOPER | W14 | Webb | NEG | NEG |
| Jan-10 | WTD | COOPER | W15 | Webb | NEG | NEG |
| Jan-10 | WTD | COOPER | W16 | Webb | NEG | NEG |
| Jan-10 | WTD | COOPER | W17 | Webb | NEG | NEG |
| Jan-10 | WTD | COOPER | W19 | Webb | NEG | NEG |
| Jan-10 | WTD | COOPER | W20 | Webb | NEG | NEG |
| Jan-10 | WTD | COOPER | W21 | Webb | NEG | NEG |
| Jan-10 | WTD | COOPER | W23 | Webb | NEG | POS |
| Jan-10 | WTD | COOPER | W24 | Webb | NEG | NEG |
| Jan-10 | WTD | COOPER | W25 | Webb | NEG | POS |
| May-10 | WTD | COOPER | L1 | Webb | NEG | NEG |
| May-10 | WTD | COOPER | L2 | Webb | POS | NEG |
| May-10 | WTD | COOPER | L3 | Webb | POS | NEG |
| May-10 | WTD | COOPER | L4 | Webb | NEG | NEG |
| May-10 | WTD | COOPER | L5 | Webb | NEG | NEG |
| May-10 | WTD | COOPER | L6 | Webb | POS | NEG |
| May-10 | WTD | COOPER | L7 | Webb | NEG | NEG |
| May-10 | WTD | COOPER | L8 | Webb | NEG | NEG |
| May-10 | WTD | COOPER | L9 | Webb | NEG | POS |
| May-10 | WTD | COOPER | L12 | Webb | POS | NEG |
| May-10 | WTD | COOPER | L13 | Webb | NEG | NEG |
| May-10 | WTD | COOPER | L14 | Webb | POS | NEG |
| May-10 | WTD | COOPER | L15 | Webb | NEG | NEG |
| May-10 | WTD | COOPER | L16 | Webb | NEG | NEG |
| May-10 | WTD | COOPER | L17 | Webb | POS | NEG |
| May-10 | WTD | COOPER | L18 | Webb | POS | NEG |
| May-10 | WTD | COOPER | L19 | Webb | POS | NEG |
| May-10 | WTD | COOPER | L20 | Webb | NEG | NEG |

**Table 11.** Continued

| Date (Month/Year) | Species | Source | Animal ID | County | *Trypanosoma* sp | *Babesia* sp |
|---|---|---|---|---|---|---|
| May-10 | WTD | COOPER | L21 | Webb | POS | NEG |
| May-10 | WTD | COOPER | L23 | Webb | NEG | NEG |
| May-10 | WTD | COOPER | L25 | Webb | POS | NEG |
| May-10 | WTD | COOPER | L26 | Webb | NEG | NEG |
| May-10 | WTD | COOPER | L27 | Webb | POS | NEG |
| May-10 | WTD | COOPER | L28 | Webb | NEG | POS |
| May-10 | WTD | COOPER | L30 | Webb | POS | NEG |
| May-10 | WTD | COOPER | L31 | Webb | POS | NEG |
| May-10 | WTD | COOPER | L32 | Webb | POS | NEG |
| May-10 | WTD | COOPER | L33 | Webb | NEG | NEG |
| May-10 | WTD | COOPER | L34 | Webb | POS | NEG |
| May-10 | WTD | COOPER | L36 | Webb | NEG | NEG |
| May-10 | WTD | COOPER | L37 | Webb | POS | NEG |
| May-10 | WTD | COOPER | L38 | Webb | NEG | NEG |
| May-10 | WTD | COOPER | L40 | Webb | NEG | NEG |
| **Total Positive (% Positive)** | | | | | **18 (35.2%)** | **4 (7.8%)** |

| Date (Month/Year) | Species | Source | Animal ID | County | *Trypanosoma* sp | *Babesia* sp |
|---|---|---|---|---|---|---|
| *WTD in Zapata County* | | | | | | |
| Jul-10 | WTD | SCHUSTER | NL1 | Zapata | POS | NEG |
| Jul-10 | WTD | SCHUSTER | NL2 | Zapata | POS | NEG |
| Jul-10 | WTD | SCHUSTER | NL3 | Zapata | POS | NEG |
| Jul-10 | WTD | SCHUSTER | NL4 | Zapata | POS | NEG |
| Jul-10 | WTD | SCHUSTER | NL5 | Zapata | NEG | NEG |
| Jul-10 | WTD | SCHUSTER | NL6 | Zapata | POS | NEG |
| Jul-10 | WTD | SCHUSTER | NL7 | Zapata | POS | NEG |
| Jul-10 | WTD | SCHUSTER | NL8 | Zapata | NEG | NEG |
| Jul-10 | WTD | SCHUSTER | NL9 | Zapata | POS | NEG |
| Jul-10 | WTD | SCHUSTER | NL10 | Zapata | POS | NEG |
| Jul-10 | WTD | SCHUSTER | NL11 | Zapata | POS | NEG |
| Jul-10 | WTD | SCHUSTER | NL12 | Zapata | POS | NEG |
| Jul-10 | WTD | SCHUSTER | NL13 | Zapata | POS | NEG |
| Jul-10 | WTD | SCHUSTER | NL14 | Zapata | POS | NEG |
| Jul-10 | WTD | SCHUSTER | NL15 | Zapata | POS | NEG |
| Jul-10 | WTD | SCHUSTER | SL4 | Zapata | POS | NEG |
| Jul-10 | WTD | SCHUSTER | SL5 | Zapata | NEG | NEG |

**Table 11.** Continued

| Date (Month/Year) | Species | Source | Animal ID | County | *Trypanosoma* sp | *Babesia* sp |
|---|---|---|---|---|---|---|
| Jul-10 | WTD | SCHUSTER | SL6 | Zapata | NEG | NEG |
| Jul-10 | WTD | SCHUSTER | SL7 | Zapata | NEG | NEG |
| Jul-10 | WTD | SCHUSTER | SL8 | Zapata | NEG | NEG |
| Jul-10 | WTD | SCHUSTER | SL9 | Zapata | NEG | NEG |
| Jul-10 | WTD | SCHUSTER | SL12 | Zapata | NEG | NEG |
| Jul-10 | WTD | SCHUSTER | SL13 | Zapata | NEG | NEG |
| Jul-10 | WTD | SCHUSTER | SL14 | Zapata | NEG | NEG |
| Jul-10 | WTD | SCHUSTER | SL15 | Zapata | NEG | NEG |
| Jul-10 | WTD | SCHUSTER | HF16 | Zapata | POS | NEG |
| Jul-10 | WTD | SCHUSTER | HF17 | Zapata | POS | NEG |
| Jul-10 | WTD | SCHUSTER | HF18 | Zapata | POS | NEG |
| Jul-10 | WTD | SCHUSTER | HF19 | Zapata | POS | NEG |
| Jul-10 | WTD | SCHUSTER | HF20 | Zapata | NEG | NEG |
| Jul-10 | WTD | SCHUSTER | HF24 | Zapata | NEG | NEG |
| Jul-10 | WTD | SCHUSTER | HF25 | Zapata | POS | NEG |
| Jul-10 | WTD | SCHUSTER | HF27 | Zapata | POS | NEG |
| Jul-10 | WTD | SCHUSTER | HF28 | Zapata | POS | NEG |
| Jul-10 | WTD | SCHUSTER | HF29 | Zapata | NEG | NEG |
| Aug-10 | WTD | COOPER | R2 | Zapata | NEG | NEG |
| Aug-10 | WTD | COOPER | R8 | Zapata | NEG | NEG |
| Aug-10 | WTD | COOPER | R11 | Zapata | NEG | NEG |
| Aug-10 | WTD | COOPER | R23 | Zapata | NEG | NEG |
| Aug-10 | WTD | COOPER | R25 | Zapata | NEG | NEG |
| Aug-10 | WTD | COOPER | R31 | Zapata | NEG | NEG |
| Aug-10 | WTD | COOPER | R32 | Zapata | NEG | NEG |
| Aug-10 | WTD | COOPER | R35 | Zapata | NEG | NEG |
| Aug-10 | WTD | COOPER | Y15 | Zapata | NEG | NEG |
| Oct-10 | WTD | SCHUSTER | HF101 | Zapata | POS | NEG |
| Oct-10 | WTD | SCHUSTER | HF103 | Zapata | POS | NEG |
| Oct-10 | WTD | SCHUSTER | HF104 | Zapata | NEG | NEG |
| Oct-10 | WTD | SCHUSTER | HF105 | Zapata | POS | NEG |
| Oct-10 | WTD | SCHUSTER | HF106 | Zapata | POS | NEG |
| Oct-10 | WTD | SCHUSTER | HF107 | Zapata | POS | NEG |
| Oct-10 | WTD | SCHUSTER | HF108 | Zapata | NEG | NEG |
| Oct-10 | WTD | SCHUSTER | HF109 | Zapata | POS | NEG |

**Table 11.** Continued

| Date (Month/Year) | Species | Source | Animal ID | County | *Trypanosoma* sp | *Babesia* sp |
|---|---|---|---|---|---|---|
| Oct-10 | WTD | SCHUSTER | HF110 | Zapata | NEG | NEG |
| Oct-10 | WTD | SCHUSTER | HF111 | Zapata | POS | NEG |
| Oct-10 | WTD | SCHUSTER | HF113 | Zapata | NEG | NEG |
| Oct-10 | WTD | SCHUSTER | HF114 | Zapata | POS | NEG |
| Oct-10 | WTD | SCHUSTER | HF115 | Zapata | NEG | NEG |
| Oct-10 | WTD | SCHUSTER | HF116 | Zapata | NEG | NEG |
| Oct-10 | WTD | SCHUSTER | HF117 | Zapata | NEG | NEG |
| Oct-10 | WTD | SCHUSTER | HF118 | Zapata | NEG | NEG |
| Oct-10 | WTD | SCHUSTER | HF119 | Zapata | POS | NEG |
| Feb-11 | WTD | SCHUSTER | 147 | Zapata | POS | NEG |
| Feb-11 | WTD | SCHUSTER | 148 | Zapata | POS | NEG |
| Feb-11 | WTD | SCHUSTER | 149 | Zapata | POS | NEG |
| Feb-11 | WTD | SCHUSTER | 150 | Zapata | POS | NEG |
| Feb-11 | WTD | SCHUSTER | 151 | Zapata | POS | NEG |
| Feb-11 | WTD | SCHUSTER | 152 | Zapata | POS | NEG |
| Feb-11 | WTD | SCHUSTER | 153 | Zapata | POS | NEG |
| Feb-11 | WTD | SCHUSTER | 154 | Zapata | POS | NEG |
| Feb-11 | WTD | SCHUSTER | 155 | Zapata | NEG | NEG |
| Feb-11 | WTD | SCHUSTER | 156 | Zapata | POS | NEG |
| Feb-11 | WTD | SCHUSTER | 157 | Zapata | POS | NEG |
| Feb-11 | WTD | SCHUSTER | 158 | Zapata | POS | NEG |
| Feb-11 | WTD | SCHUSTER | 159 | Zapata | POS | NEG |
| Feb-11 | WTD | SCHUSTER | 160 | Zapata | POS | NEG |
| Feb-11 | WTD | SCHUSTER | 161 | Zapata | POS | NEG |
| Feb-11 | WTD | SCHUSTER | 162 | Zapata | POS | NEG |
| Feb-11 | WTD | SCHUSTER | 163 | Zapata | POS | NEG |
| May-11 | WTD | SCHUSTER | A1 | Zapata | POS | NEG |
| May-11 | WTD | SCHUSTER | A2 | Zapata | POS | NEG |
| May-11 | WTD | SCHUSTER | A3 | Zapata | POS | POS |
| May-11 | WTD | SCHUSTER | A4 | Zapata | POS | NEG |
| May-11 | WTD | SCHUSTER | A5 | Zapata | POS | NEG |
| May-11 | WTD | SCHUSTER | A6 | Zapata | NEG | NEG |
| May-11 | WTD | SCHUSTER | A7 | Zapata | POS | NEG |
| May-11 | WTD | SCHUSTER | A8 | Zapata | POS | NEG |
| May-11 | WTD | SCHUSTER | A9 | Zapata | NEG | NEG |
| May-11 | WTD | SCHUSTER | A10 | Zapata | POS | NEG |
| May-11 | WTD | SCHUSTER | A11 | Zapata | NEG | NEG |

**Table 11.** Continued

| **Date (Month/Year) Species** | **Source** | **Animal ID** | **County** | ***Trypanosoma* sp** | ***Babesia* sp** | |
|---|---|---|---|---|---|---|
| May-11 | WTD | SCHUSTER | A13 | Zapata | NEG | NEG |
| May-11 | WTD | SCHUSTER | A14 | Zapata | NEG | NEG |
| May-11 | WTD | SCHUSTER | A15 | Zapata | POS | NEG |
| May-11 | WTD | SCHUSTER | A16 | Zapata | POS | NEG |
| May-11 | WTD | SCHUSTER | A17 | Zapata | NEG | NEG |
| May-11 | WTD | SCHUSTER | A18 | Zapata | NEG | NEG |
| May-11 | WTD | SCHUSTER | A19 | Zapata | NEG | NEG |
| May-11 | WTD | SCHUSTER | A20 | Zapata | POS | NEG |
| May-11 | WTD | SCHUSTER | A21 | Zapata | POS | POS |
| May-11 | WTD | SCHUSTER | A22 | Zapata | POS | POS |
| May-11 | WTD | SCHUSTER | A23 | Zapata | NEG | NEG |
| May-11 | WTD | SCHUSTER | A24 | Zapata | POS | NEG |
| May-11 | WTD | SCHUSTER | A25 | Zapata | NEG | NEG |
| May-11 | WTD | SCHUSTER | A26 | Zapata | NEG | NEG |
| May-11 | WTD | SCHUSTER | A27 | Zapata | NEG | NEG |
| May-11 | WTD | SCHUSTER | A28 | Zapata | POS | NEG |
| May-11 | WTD | SCHUSTER | A29 | Zapata | POS | NEG |
| May-11 | WTD | SCHUSTER | A30 | Zapata | NEG | NEG |
| May-11 | WTD | SCHUSTER | A31 | Zapata | POS | NEG |
| May-11 | WTD | SCHUSTER | A32 | Zapata | NEG | NEG |
| May-11 | WTD | SCHUSTER | A33 | Zapata | NEG | POS |
| May-11 | WTD | SCHUSTER | A34 | Zapata | NEG | NEG |
| May-11 | WTD | SCHUSTER | A35 | Zapata | NEG | NEG |
| **Total Positive (% Positive)** | | | | | **64 (56%)** | **4 (3.5%)** |
| **Date (Month/Year)** | **Species** | **Source** | **Animal ID** | **County** | ***Trypanosoma* sp** | ***Babesia* sp** |
| *WTD in Starr County* | | | | | | |
| Sep-11 | WTD | COOPER | S1 | Starr | POS | NEG |
| Sep-11 | WTD | COOPER | S2 | Starr | POS | NEG |
| Sep-11 | WTD | COOPER | S3 | Starr | POS | NEG |
| Sep-11 | WTD | COOPER | S4 | Starr | POS | NEG |
| Sep-11 | WTD | COOPER | S5 | Starr | NEG | NEG |
| Sep-11 | WTD | COOPER | S6 | Starr | POS | NEG |
| Sep-11 | WTD | COOPER | S7 | Starr | POS | NEG |

**Table 11.** Continued

| Date (Month/Year) | Species | Source | Animal ID | County | *Trypanosoma* sp | *Babesia* sp |
|---|---|---|---|---|---|---|
| Sep-11 | WTD | COOPER | S8 | Starr | POS | NEG |
| Sep-11 | WTD | COOPER | S9 | Starr | POS | NEG |
| Sep-11 | WTD | COOPER | S11 | Starr | POS | NEG |
| Sep-11 | WTD | COOPER | S12 | Starr | POS | NEG |
| Sep-11 | WTD | COOPER | S13 | Starr | POS | NEG |
| Sep-11 | WTD | COOPER | S14 | Starr | POS | NEG |
| Sep-11 | WTD | COOPER | S15 | Starr | POS | NEG |
| Sep-11 | WTD | COOPER | S17 | Starr | POS | NEG |
| Sep-11 | WTD | COOPER | S18 | Starr | POS | NEG |
| Sep-11 | WTD | COOPER | S19 | Starr | POS | NEG |
| Sep-11 | WTD | COOPER | S20 | Starr | POS | NEG |
| Sep-11 | WTD | COOPER | S21 | Starr | POS | NEG |
| Sep-11 | WTD | COOPER | S22 | Starr | POS | NEG |
| Sep-11 | WTD | COOPER | S23 | Starr | POS | NEG |
| Sep-11 | WTD | COOPER | S24 | Starr | POS | NEG |
| Sep-11 | WTD | COOPER | S25 | Starr | POS | NEG |
| Sep-11 | WTD | COOPER | S26 | Starr | POS | NEG |
| Sep-11 | WTD | COOPER | S27 | Starr | POS | NEG |
| Sep-11 | WTD | COOPER | S28 | Starr | POS | NEG |
| Sep-11 | WTD | COOPER | S29 | Starr | POS | NEG |
| Sep-11 | WTD | COOPER | S30 | Starr | POS | NEG |
| Sep-11 | WTD | COOPER | S31 | Starr | POS | NEG |
| Sep-11 | WTD | COOPER | S32 | Starr | POS | NEG |
| Sep-11 | WTD | COOPER | S33 | Starr | NEG | NEG |
| Sep-11 | WTD | COOPER | S34 | Starr | POS | NEG |
| Sep-11 | WTD | COOPER | S35 | Starr | POS | NEG |
| Sep-11 | WTD | COOPER | S36 | Starr | POS | NEG |
| Sep-11 | WTD | COOPER | S37 | Starr | POS | NEG |
| Sep-11 | WTD | COOPER | S38 | Starr | NEG | NEG |
| Sep-11 | WTD | COOPER | S39 | Starr | POS | NEG |
| Sep-11 | WTD | COOPER | S40 | Starr | POS | NEG |
| Sep-11 | WTD | COOPER | S41 | Starr | POS | NEG |
| Sep-11 | WTD | COOPER | S42 | Starr | POS | NEG |
| Sep-11 | WTD | COOPER | S43 | Starr | POS | NEG |
| Sep-11 | WTD | COOPER | S44 | Starr | POS | NEG |
| Sep-11 | WTD | COOPER | S45 | Starr | POS | NEG |
| Sep-11 | WTD | COOPER | S46 | Starr | POS | NEG |

**Table 11.** Continued

| Date (Month/Year) | Species | Source | Animal ID | County | *Trypanosoma* sp | *Babesia* sp |
|---|---|---|---|---|---|---|
| Sep-11 | WTD | COOPER | S47 | Starr | POS | NEG |
| Sep-11 | WTD | COOPER | S48 | Starr | POS | NEG |
| Sep-11 | WTD | COOPER | S49 | Starr | POS | NEG |
| Sep-11 | WTD | COOPER | S51 | Starr | POS | NEG |
| Sep-11 | WTD | COOPER | S52 | Starr | POS | NEG |
| Sep-11 | WTD | COOPER | S53 | Starr | POS | NEG |
| Sep-11 | WTD | COOPER | S54 | Starr | POS | NEG |
| Sep-11 | WTD | COOPER | S55 | Starr | POS | NEG |
| Sep-11 | WTD | COOPER | S56 | Starr | POS | NEG |
| Sep-11 | WTD | COOPER | S57 | Starr | POS | NEG |
| Sep-11 | WTD | COOPER | S58 | Starr | POS | NEG |
| Sep-11 | WTD | COOPER | S59 | Starr | POS | NEG |
| Sep-11 | WTD | COOPER | S60 | Starr | NEG | NEG |
| Sep-11 | WTD | COOPER | S61 | Starr | POS | NEG |
| Sep-11 | WTD | COOPER | S62 | Starr | POS | NEG |
| Sep-11 | WTD | COOPER | S63 | Starr | POS | NEG |
| Sep-11 | WTD | COOPER | S64 | Starr | NEG | NEG |
| Sep-11 | WTD | COOPER | S66 | Starr | POS | NEG |
| Sep-11 | WTD | COOPER | S67 | Starr | POS | NEG |
| Sep-11 | WTD | COOPER | S68 | Starr | POS | NEG |
| Sep-11 | WTD | COOPER | S69 | Starr | POS | NEG |
| Sep-11 | WTD | COOPER | S70 | Starr | POS | NEG |
| Sep-11 | WTD | COOPER | S71 | Starr | POS | NEG |
| Sep-11 | WTD | COOPER | S72 | Starr | NEG | NEG |
| Sep-11 | WTD | COOPER | S73 | Starr | POS | NEG |
| Sep-11 | WTD | COOPER | S74 | Starr | NEG | NEG |
| Sep-11 | WTD | COOPER | S75 | Starr | POS | NEG |
| Sep-11 | WTD | COOPER | S76 | Starr | POS | NEG |
| Sep-11 | WTD | COOPER | S77 | Starr | POS | NEG |
| Sep-11 | WTD | COOPER | S78 | Starr | NEG | NEG |
| Sep-11 | WTD | COOPER | S79 | Starr | POS | NEG |
| Sep-11 | WTD | COOPER | S80 | Starr | NEG | NEG |
| Sep-11 | WTD | COOPER | S81 | Starr | NEG | NEG |
| Sep-11 | WTD | COOPER | S82 | Starr | NEG | NEG |
| Sep-11 | WTD | COOPER | No # | Starr | POS | NEG |
| **Total Positive (% Positive)** | | | | | **70 (86.4%)** | **0 (0%)** |
| **Grand Total WTD positives (all counties)** | | | | | **152 (62%)** | **8 (3.26%)** |

**Table 12.** Cattle PCR results for *Trypanosoma* sp. and *Babesia* sp. in Starr County

| Date (Month/Year) | Host | Source | Animal ID | County | *Trypanosoma* sp | *Babesia* sp |
|---|---|---|---|---|---|---|
| *Cattle in Starr County* | | | | | | |
| Mar-10 | Cattle | COOPER | 4 2 | Starr | NEG | NEG |
| Mar-10 | Cattle | COOPER | 2 | Starr | POS | NEG |
| Mar-10 | Cattle | COOPER | 4 | Starr | NEG | NEG |
| Mar-10 | Cattle | COOPER | 12 | Starr | NEG | NEG |
| Mar-10 | Cattle | COOPER | 24 | Starr | NEG | NEG |
| Mar-10 | Cattle | COOPER | 31 | Starr | NEG | NEG |
| Mar-10 | Cattle | COOPER | 34 | Starr | NEG | NEG |
| Mar-10 | Cattle | COOPER | 36 | Starr | NEG | NEG |
| Mar-10 | Cattle | COOPER | 45 | Starr | NEG | NEG |
| Mar-10 | Cattle | COOPER | 46 | Starr | NEG | NEG |
| Mar-10 | Cattle | COOPER | 47 | Starr | NEG | NEG |
| Mar-10 | Cattle | COOPER | 48 | Starr | NEG | NEG |
| Mar-10 | Cattle | COOPER | 56 | Starr | NEG | NEG |
| Mar-10 | Cattle | COOPER | 60 | Starr | POS | NEG |
| Mar-10 | Cattle | COOPER | 63 | Starr | NEG | NEG |
| Mar-10 | Cattle | COOPER | 66 | Starr | POS | NEG |
| Mar-10 | Cattle | COOPER | 67 | Starr | NEG | NEG |
| Mar-10 | Cattle | COOPER | 73 | Starr | NEG | NEG |
| Mar-10 | Cattle | COOPER | 95 | Starr | NEG | NEG |
| Mar-10 | Cattle | COOPER | 96 | Starr | NEG | NEG |
| Mar-10 | Cattle | COOPER | 104 | Starr | POS | NEG |
| Mar-10 | Cattle | COOPER | 107 | Starr | POS | NEG |
| Mar-10 | Cattle | COOPER | 117 | Starr | NEG | NEG |
| Mar-10 | Cattle | COOPER | 128 | Starr | NEG | NEG |
| Mar-10 | Cattle | COOPER | 130 | Starr | NEG | NEG |
| Mar-10 | Cattle | COOPER | 133 | Starr | POS | NEG |
| Mar-10 | Cattle | COOPER | 139 | Starr | POS | NEG |
| Mar-10 | Cattle | COOPER | 165 | Starr | NEG | NEG |
| Mar-10 | Cattle | COOPER | 169 | Starr | NEG | NEG |
| Mar-10 | Cattle | COOPER | 196 | Starr | POS | NEG |
| Mar-10 | Cattle | COOPER | 219 | Starr | POS | NEG |
| Mar-10 | Cattle | COOPER | 2031 | Starr | NEG | NEG |
| Mar-10 | Cattle | COOPER | 2048 | Starr | NEG | NEG |
| Mar-10 | Cattle | COOPER | 2068 | Starr | NEG | NEG |
| Mar-10 | Cattle | COOPER | 2070 | Starr | NEG | NEG |

**Table 12** continued

| Date (Month/Year) | Host | Source | Animal ID | County | *Trypanosoma* sp | *Babesia* sp |
|---|---|---|---|---|---|---|
| Mar-10 | Cattle | COOPER | 2073 | Starr | POS | NEG |
| Mar-10 | Cattle | COOPER | 2095 | Starr | POS | NEG |
| Mar-10 | Cattle | COOPER | 2110 | Starr | NEG | NEG |
| Mar-10 | Cattle | COOPER | 2112 | Starr | NEG | NEG |
| Mar-10 | Cattle | COOPER | 2124 | Starr | POS | NEG |
| Mar-10 | Cattle | COOPER | 2125 | Starr | NEG | NEG |
| Mar-10 | Cattle | COOPER | 2127 | Starr | POS | NEG |
| Mar-10 | Cattle | COOPER | 2166 | Starr | NEG | NEG |
| Mar-10 | Cattle | COOPER | 2176 | Starr | NEG | NEG |
| Mar-10 | Cattle | COOPER | 2186 | Starr | NEG | NEG |
| Mar-10 | Cattle | COOPER | 2201 | Starr | POS | NEG |
| Mar-10 | Cattle | COOPER | 3005 | Starr | NEG | NEG |
| Mar-10 | Cattle | COOPER | 3006 | Starr | NEG | NEG |
| Mar-10 | Cattle | COOPER | 3023 | Starr | NEG | NEG |
| Mar-10 | Cattle | COOPER | 3024 | Starr | NEG | NEG |
| Mar-10 | Cattle | COOPER | 3026 | Starr | POS | NEG |
| Mar-10 | Cattle | COOPER | 3040 | Starr | NEG | NEG |
| Mar-10 | Cattle | COOPER | 3071 | Starr | POS | NEG |
| Mar-10 | Cattle | COOPER | 3099 | Starr | POS | NEG |
| Mar-10 | Cattle | COOPER | 3135 | Starr | POS | NEG |
| Mar-10 | Cattle | COOPER | 3520 | Starr | NEG | NEG |
| Mar-10 | Cattle | COOPER | 3535 | Starr | POS | NEG |
| Mar-10 | Cattle | COOPER | 3539 | Starr | POS | NEG |
| Mar-10 | Cattle | COOPER | 3577 | Starr | POS | NEG |
| Mar-10 | Cattle | COOPER | 3635 | Starr | POS | NEG |
| Mar-10 | Cattle | COOPER | 4253 | Starr | POS | NEG |
| Mar-10 | Cattle | COOPER | 9025 | Starr | NEG | NEG |
| Mar-10 | Cattle | COOPER | 9082 | Starr | NEG | NEG |
| Mar-10 | Cattle | COOPER | 9311 | Starr | NEG | NEG |
| **Total Positive (% Positive)** | | | | | **23 (35.3%)** | **0 (0%)** |

Webb County had the lowest incidence with 35.2% (18/51) positive by *Trypanosoma* 18S rDNA PCR. Webb County also had the highest incidence of *Babesia* with 7.8% (4/51) of samples with positive bands on 18S rDNA PCR. Zapata County was in the middle with *Trypanosoma* occurrence at 56% (64/113) and *Babesia* at 3.5% (4/113) incidence. Overall, of a total of 245 WTD tested, 152 were positive for *Trypanosoma* (62%) and 8 were positive for *Babesia* spp. (3.26%).

The cattle samples were obtained from Starr County and the *Babesia* occurrence was 0% (0/65) while the *Trypanosoma* occurrence was at 35.3% (23/65) (Table 12). The $\chi^2$ test did not find an association between *Trypanosome* and *Babesia*-infected WTD in any of the counties listed ($\alpha$ = 0.05: Pearson probability for Webb county = 0.1239, for Zapata = 0.4505, and for Starr = 0 since no *Babesia* present).

The months that the WTD blood samples were collected were broken into the following four groups based on average temperatures and average rainfall for south western Texas taking into account the celestial calendar, December to February, March to May, June to August, and September to November. Cattle samples were only taken during March to May season and reported in that season. During the first season of December to February, 51% (18/35) of the total samples taken during that season were positive for *Trypanosoma* and 5.7% (2/35) were positive for *Babesia.* In the second season of March to May, 23.3% (31/133) WTD samples taken were positive for *Trypanosoma* and 35.3% (23/65) of the cattle samples; 3% (4/133) WTD samples were positive for *Babesia* and 0% (0/65) cattle samples. In the third season of June to August, 47.7% (21/44) samples were positive for *Trypanosoma* and 0% (0/44) were positive for *Babesia.* The last season of September to November found 80% (79/98) positive for *Trypanosoma* and 2% (2/98) positive for *Babesia.* The $\chi^2$ test found none of the seasons to have a correlation between *Trypanosoma* and *Babesia* (Pearson probability with $\alpha = 0.05$: December to February = 0.1340, March to May = 0.6636, June to August and September to November had degrees of freedom = 0 with no probability).

**DISCUSSION**

*Babesia bovis* is transmitted by the cattle fever ticks (*Rhipicephalus (Boophilus) annulatus* and *Rhipicephalus (Boophilus) microplus)*. These ticks have been eradicated from the United States and border surveillance still exists that controls the movement of animals (mostly cattle) across from Mexico into Texas. It is possible that WTD are able to travel back and forth over the border effectively transmitting the tick from Mexico to the United States. This possibility is small, but still possible especially considering the sampled animals lived in Texas counties that border Mexico. *Trypanosoma theileri* has been found to be transmitted by the tabanid fly, while the ked (*Lipoptena mazamae)* has been implicated in the transmission of *Trypanosoma cervi*.

Blood samples were collected from cattle and WTD from different counties in South Texas at different times of the year and an 18S rDNA screening PCR was done for both *B. bovis* and *Trypanosoma.* The current study compared the *Trypanosoma* and *Babesia* found in both cattle and WTD to the region where the parasite was found and the season it during which it was found.

It is possible that *Trypanosoma* and *Babesia* blood parasites compete within the mammalian host and that is why the Texas county of Starr, with the highest *Trypanosoma* spp. parasites also had the lowest *Babesia* spp. parasites and Webb County had the reverse with the highest occurrence of *Babesia* spp. and the lowest incidence of *Trypanosoma* spp. The $\chi^2$ test did not show an association between the two parasites in the different counties. It could be that the observations made during this study are not representative and the $\chi^2$ test was true, there was not an association and therefore no competition between the parasites in the same hosts.

Within the four seasonal periods of December to February, March to May, June to August, and September to November, the highest occurrence of *Trypanosoma* spp. was during September to November (80%) and the lowest occurrence was March to May (23.3%). For *Babesia* sp. the seasonal group with the highest occurrence was during December to February (5.7%) and the lowest occurrence was during June to August (0%). The *R. annulatus* and *R. microplus* ticks which transmit *Babesia* tend to feed more often in hotter, dryer summer months, but desiccation risk is greater in hot dry environments, which could be why the highest occurrence was seen December to February and the lowest in the hot months of June to August. Previous studies have not determined without a doubt that the *Babesia* found in WTD is the same *B. bovis* found in cattle (Cantu et al., 2007; Holman et al., 2010; Ramos et al., 2010). It could be that the *Babesia* found in this study are not *B. bovis* and are transmitted by a different tick vector.

The tabanid fly has been found to transmit *T. theileri* in cattle (Foil and Hogsette, 1994). This fly is generally found more often during warm months, which does not correlate perfectly with the incidence seen for *Trypanosoma* in WTD and cattle in this study. If the tabanid fly only transmits *T. theileri* more cattle samples would need to be taken to determine whether there is a correlation between vector prevalence and infection occurrence. Previously it was reported that the incidence of the ked, *L. mazamae* that potentially transmits *T. cervi* to WTD in south Florida was lower in the month of October than in the months of March, June, and August (Samuel et al., 2001). This does not appear to correlate with the results of the current study, which found the highest occurrence of *Trypanosoma* in WTD during September to November. The previous study (Samuel et al., 2001) also suggested that the increase of rain may increase the numbers of this ked. In September of 2011, when one batch of samples was taken, the National Climate Data Center reported above average temperatures for South Texas during this month and much below normal precipitation. In October of 2010, when the other batch of samples was taken the National Climate Data Center reported below normal temperatures and much below normal precipitation (National Climate Data Center, http://www.ncdc.noaa.gov/oa/ncdc.html). It may be that in Texas the ked is more prevalent during warmer times of the year and amount of precipitation does not affect the ked. It could also be that the ked is not the vector for *T. cervi.*

In conclusion, looking at the percentages of each parasite found in each county, it appears a correlation could be made and that where a high occurrence of one parasite was found a low occurrence of the other was noted. Based on the $\chi^2$ tests done statistically, though, there is no significant correlation between *Trypanosoma* and *Babesia* in the three South Texas counties of Starr, Zapata, and Webb. The correlation between positive samples and seasonal effects could not be statistically confirmed, but it appears that *Babesia* infected animals are found in lowest numbers during hot, dry seasons. The studies done previously in other areas on ked and tabanid fly prevalence did not appear to correlate with the findings of *Trypanosoma* in the present study. It could be that Texas has different tabanid and ked fly seasons or the *Trypanosoma* found in the present study is transmitted by more than one insect vector.

# CHAPTER VI

# SUMMARY

The research conducted for this thesis focused on both *Babesia* and *Trypanosoma* and the culturing and molecular identification of these parasites from different hosts. Amplification of the *Babesia bovis* small subunit ribosomal RNA gene identified WTD in two southern counties of Texas as possible carriers of this parasite. The sequence analysis of the gene was unable to indisputably demonstrate the presence of this parasite in WTD and the WTD samples cultured in this study unfortunately did not produce a detectable *Babesia* parasitemia in in vitro culture. No PCR positives were found in the cattle samples. For future studies, sequencing and analyzing other regions of the ribosomal genes, such as the ITS1, 5.8S, and ITS2 regions would give a clearer picture of the *Babesia* parasites involved. Positive samples from WTD will also need to be cultured and have growth of *Babesia* spp. in order to visualize and morphologically compare this species with the *B. bovis* obtained from cattle.

This study found very close phylogenetic relationships between the WTD and elk trypanosome ribosomal DNA sequences. The phylogenies based on the ITS1-5.8S-ITS2 genomic region and the longer 18S rDNA sequence clearly separated the cattle parasites from the WTD and elk, with the exception of one WTD and two elk samples. Thus, this study suggests that the cattle parasite sequences in this study are from different species of *Trypanosoma* from those in WTD and elk, and that the cattle sequences represent *T. theileri* and the WTD and elk sequences represent *T. cervi*. It is also possible, however, that the same species infects cattle, elk, and WTD, and that different allele sequences were obtained in this study. This could mean that there are additional *Trypanosoma* species that can infect cattle and wild ungulates, or that the different parasite species can cross from wild ungulates to cattle and vice versa. Overall, the cattle sequences aligned with cattle isolates and the WTD sequences with elk sequences, indicating that wild ungulates (WTD and elk) and cattle most likely have separate trypanosome species.

The *Trypanosoma* cultured in the current study best resembles *T.cervi* with a large nucleus, free flagellum, pronounced undulating membrane, and kinetoplast located posterior to the nucleus as described by Kingston and Morton (1975). *T. theileri* has a kinetoplast that is further away from the nucleus than in the *Trypanosoma* trypomastigote imaged from the WTD culture, and the undulating membrane is shorter and more tightly adhered to the anterior end. Morphologically, the parasite cultured from WTD in this study appears to be *T. cervi*. The current study used normal adult bovine serum, fetal bovine serum, or WTD serum in HL-1 medium supplemented with l-glutamine and antibiotic-antimycotic. The parasites only grew in the medium that had FBS as the serum source, rather than with the bovine or the WTD serum. FBS supplementation of a serum-free medium designed for blood cell culture supported the in vitro propagation of the *Trypanosoma* sp from WTD in this study. The current study also compared the *Trypanosoma* and *Babesia* found in both cattle and WTD to the region where the parasite was found and the season during which it was found. It is possible that *Trypanosoma* and *Babesia* blood parasites compete within the mammalian host and that is why the Texas county of Starr, with the highest percentage of WTD infected with*Trypanosoma* spp. parasites also had the lowest percentage of WTD infected with *Babesia* spp. parasites and Webb County had the reverse with the highest occurrence of *Babesia* spp. and the lowest incidence of *Trypanosoma* spp. The $\chi^2$ test did not show an association between the two parasites in the different counties. The percentages of each parasite found in each county appear to show a correlation that where a high occurrence of one parasite was found a low occurrence of the other was noted. Within the four seasonal periods of December to February, March to May, June to August, and September to November, the highest occurrence of *Trypanosoma* spp. was during September to November (80%) and the lowest occurrence was March to May (40.6%). The studies done previously in other areas on ked and tabanid fly prevalence did not appear to correlate with the findings of *Trypanosoma* in the present study. It could be that Texas has different tabanid and ked fly seasons or the *Trypanosoma* found in the present study is not transmitted by either of these two flies. For *Babesia* sp. the seasonal group with the highest occurrence was during December to February (5.7%) and the lowest occurrence was during June to August (0%). The correlation between positive samples and seasonal effects could not be statistically confirmed, but it appears that *Babesia* infected animals are found in lowest numbers during hot, dry seasons.

Based on morphological distinctions and genomic sequencing, it appears that wild ungulates (specifically WTD) have a different *Trypanosoma* species from cattle. It is most likely that cattle are infected with *T. theileri* and wild ungulates are infected with *T. cervi.* This study was unable to unequivocally determine whether cattle *B. bovis* infect WTD. It also appears that *Trypanosoma* may be able to outcompete *Babesia* in WTD in the three South Texas counties sampled in this study. More research is needed in the area of WTD *Babesia.* This study's findings and the *Trypanosoma* sequences generated from this project will aid in future diagnostic and epidemiologic studies. The sequences have been submitted to GenBank and therefore have been made available for public use.

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# APPENDIX A

CLUSTALW of 18S rRNA gene

| | | |
|---|---|---|
| AJ009164Tth | **G**ACGTTCTCTGTTCCGGCGGTGGGGCAACTCACTGTCATGGG**A**CGTCCAGCGAATGAATG | 60 |
| Cow2073cl7 | **G**ACGTTCTCTGTTCCGGCGGTGGGGCAACTCACTGTCATGGG**A**CGTCCAGCGAATGAATG | 60 |
| AJ009163Tth | GACGTTCTCTGTTCCGGCGGTGGGGCAACTCACTGTCATGGG**A**CGTCCAGCGAATGAATG | 60 |
| Cow2073cl9 | GACGTTCTCTGTTCCGGCGGTGGGGCAACTCACTGTCATGGG**A**CGTCCAGCGAATGAATG | 60 |
| AJ009165TspCd | GACGTTCTCTGTTCCGGCGGTGGGGCAACTCACTGTCATGGGGCGTCCAGCGAATGAATG | 60 |
| WTDA21cl6 | GACGTTCTCTGTTCCGGCGGTGGGGCAACTCACTGTCATGGGGCGTCCAGCGAATGAATG | 60 |
| WTDA5cl1 | GACGTTCTCTGTTCCGGCGGTGGGGCAACTCACTGTCATGGGGCGTCCAGCGAATGAATG | 60 |
| WTDA1Cl1 | GACGTTCTCTGTTCCGGCGGTGGGGCAACTCACTGTCATGGGGCGTCCAGCGAATGAATG | 60 |
| MNelk328cl21 | GACGTTCTCTGTTCCGGCGGTGGGGCAACTCACTGTCATGGG**A**CGTCCAGCGAATGAATG | 60 |
| WTD148cl4 | GACGTTCTCTGTTCCGGCGGTGGGGCAACTCACTGTCATGGGGCGTCCAGCGAATGAATG | 60 |
| Cow138cl2 | GACGTTCTCTGTTCCGGCGGTGGGGCAACTCACTGTCATGGGGCGTCCAGCGAATGAATG | 60 |
| WTD21cl4 | GACGTTCTCTGTTCCGGCGGTGGGGCAACTCACTGTCATGGGGCGTCCAGCGAATGAATG | 60 |
| Cow138cl4 | GACGTTCTCTGTTCCGGCGGTGGGGCAACTCACTGTCATGGGGCGTCCAGCGAATGAATG | 60 |
| WTDA1cl4 | GACGTTCTCTGTTCCGGCGGTGGGGCAACTCACTGTCATGGGGCGTCCAGCGAATGAATG | 60 |
| WTDA21cl4 | GACGTTCTCTGTTCCGGCGGTGGGGCAACTCACTGTCATGGGGCGTCCAGCGAATGAATG | 60 |
| MNelk328cl20 | GACGT**C**CTCTGTT**A**CGGCGGTGGGGCAACTCACTGTCATGGGGCGTCCAGCGAATGAATG | 60 |
| Mnelk317c4 | GACGT**C**CTCTGTT**A**CGGCGGTGGGGCAACTCACTGTCATGGGGCGTCCAGCGAATGAATG | 60 |
| Mnelk317c3 | GACGT**C**CTCTGTT**A**CGGCGGTGGGGCAACTCACTGTCATGGGGCGTCCAGCGAATGAATG | 60 |
| | ***** ******* **************************** ***************** | |

| | | |
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| Cow2073cl7 | AAAATAAAACCAATGCCTTCACCGGCAGTAACACCCAGAAGTGTTGACTCAATTCATTCC | 120 |
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| WTD148cl4 | AAATTAAAACCAATGCCTTCACCGGCAGTAACACCCAGAAGTGTTGACTCAATTCATTCC | 120 |
| Cow138cl2 | AAATTAGAACCAATGCCTTCACCGGCAGTAACGCCCAGAAGTGTTGACTCAATTCATTCC | 120 |

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WTD148cl4          TGTTAAAGGGTTCGTAGTTGAATTGTGGGCCTTTGAGGCGCAATGGTTTGTCCCGTCCAC 540
Cow138cl2          TGTTAAAGGGTTCGTAGTTGAATTGTGGGCCTTTGAGGCGCAATGGTTTGTCCCGTCCAC 540
WTD21cl4           TGTTAAAGGGTTCGTAGTTGAATTGTGGGCCTTTGAGGCGCAATGGTTTGTCCCGTCCAC 540
Cow138cl4          TGTTAAAGGGTTCGTAGTTGAATTGTGGGCCCTTGAGGCGCAATGGTTTGTCCCGTCCAC 540
WTDA1cl4           TGTTAAAGGGTTCGTAGTTGAATTGTGGGCCTTTGAGGCGCAATGGTTTGTCCCGTCCAC 540
WTDA21cl4          TGTTAAAGGGTTCGTAGTTGAATTGTGGGCCTTTGAGGCGCAATGGTTTGTCCCGTCCAC 540
MNelk328cl20       TGTTAAAGGGTTCGTAGTTGAATTGTGGGCCTTTGAGGCGCAATGGTTTGTCCCGTCCAC 540
Mnelk317c4         TGTTAAAGGGTTCGTAGTTGAATTGTGGGCCTTTGAGGCGCAATGGTTTGTCCCGTCCAC 540
Mnelk317c3         TGTTAAAGGGTTCGTAGTTGAATTGTGGGCCTTTGAGGCGCAATGGTTTGTCCCGTCCAC 540
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AJ009164Tth          CTCGGATTGGTGACCCATGCCCTTGAGGTCCGTGAACACTCAGAAACAAGAAACACGGGA 600
Cow2073cl7           CTCGGATTGGTGACCCATGCCCTTGAGGTCCGTGAACACTCAGAAACAAGAAACACGGGA 599
AJ009163Tth          CTCGGATTGGTGACCCATGCCCTTGAGGTCCGTGAACACTCAGAAACAAGAAACACGGGA 600
Cow2073cl9           CTCGGATTGGTGACCCATGCCCTTGAGGTCCGTGAACACTCAGAAACAAGAAACACGGGA 600
AJ009165TspCd        CTCGGATTGGTGACCCATGCCCTTGAGGTCCGTGAACACTCAGAAACAAGAAACACGGGA 600
WTDA21cl6            CTCGGATTGGTGACCCATGCCCTTGAGGTCCGTGAACACTCAGAAACAAGAAACACGGGA 600
WTDA5cl1             CTCGGATTGGTGACCCATGCCCTTGAGGTCCGTGAACACTCAGAAACAAGAAACACGGGA 600
WTDA1Cl1             CTCGGATTGGTGACCCATGCCCTTGAGGTCCGTGAACACTCAGAAACAAGAAACACGGGA 600
MNelk328cl21         CTCGGATTGGTGACCCATGCCCTTGAGGTCCGTGAACACTCAGAAACAAGAAACACGGGA 600
WTD148cl4            CTCGGATTGGTGACCCATGCCCTTGAGGTCCGTGAACACTCAGAAACAAGAAACACGGGA 600
Cow138cl2            CTCGGATTGGTGACCCATGCCCTTGAGGTCCGTGAACAATCAGAAACAAAAAACACGGGA 600
WTD21cl4             CTCGGATTGGTGACCCATGCCCTTGAGGTCCGTGAACAATCAGAAACAAAAAACACGGGA 600
Cow138cl4            CTCGGATTGGTGACCCATGCCCTTGAGGTCCGTGAACAATCAGAAACAAAAAACACGGGA 600
WTDA1cl4             CTCGGATTGGTGACCCATGCCCTTGAGGTCCGTGAACACTCAGAAACAAGAAACACGGGA 600
WTDA21cl4            CTCGGATTGGTGACCCATGCCCTTGAGGTCCGTGAACACTCAGAAACAAGAAACACGGGA 600
MNelk328cl20         CTCGGATTGGTGACCCATGCCCTTGAGGTCCGTGAACACTCAGAAACAAGAAACACGGGA 600
Mnelk317c4           CTCGGATTGGTGACCCATGCCCTTGAGGTCCGTGAACACTCAGAAACAAGAAACACGGGA 600
Mnelk317c3           CTCGGATTGGTGACCCATGCCCTTGAGGTCCGTGAACACTCAGAAACAAGAAACACGGGA 600
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AJ009164Tth          GCGGTTCCTTCCTGATTTTCGCATGTCATGCATGCCAGGGGGCGTCCGTGATTTTTACTG 660
Cow2073cl7           GCGGTTCCTTCCTGATTTTCGCATGTCATGCATGCCAGGGGGCGTCCGTGATTTTTACTG 659
AJ009163Tth          GCGGTTCCTTCCTGATTTTCGCATGTCATGCATGCCAGGGGGCGTCCGTGATTTTTACTG 660
Cow2073cl9           GCGGTTCCTTCCTGATTTTCGCATGTCATGCATGCCAGGGGGCGTCCGTGATTTTTACTG 660
AJ009165TspCd        GCGGTTCCTTCCTGATTTTCGCATGTCATGCATGCCAGGGGGCGTCCGTGATTTTTACTG 660
WTDA21cl6            GCGGTTCCTTCCTGATTTTCGCATGTCATGCATGCCAGGGGGCGTCCGTGATTTTTACTG 660
WTDA5cl1             GCGGTTCCTTCCTGATTTTCGCATGTCATGCATGCCAGGGGGCGTCCGTGATTTTTACTG 660
WTDA1Cl1             GCGGTTCCTTTCTGATTTTCGCATGTCATGCATGCCAGGGGGCGTCCGTGATTTTTACTG 660
MNelk328cl21         GCGGTTCCTTCCTGATTTTCGCATGTCATGCATGCCAGGGGGCGTCCGTGATTTTTACTG 660
WTD148cl4            GCGGTTCCTTCCTGATTTTCGCATGTCATGCATGCCAGGGGGCGTCCGTGATTTTTACTG 660
Cow138cl2            GCGGTTCCTTCCTGATTTTCGCATGTCATGCATGCCAGGGGGCGTCCGTGATTTTTACTG 660
WTD21cl4             GCGGTTCCTTCCTGATTTTCGCATGTCATGCATGCCAGGGGGCGTCCGTGATTTTTACTG 660
Cow138cl4            GCGGTTCCTTCCTGATTTTCGCATGTCATGCATGCCAGGGGGCGTCCGTGATTTTTACTG 660
WTDA1cl4             GCGGTTCCTTCCTGATTTTCGCATGTCATGCATGCCAGGGGGCGTCCGTGATTTTTACTG 660
WTDA21cl4            GCGGTTCCTTCCTGATTTTCGCATGTCATGCATGCCAGGGGGCGTCCGTGATTTTTACTG 660
MNelk328cl20         GCGGTTCCTTCCTGATTTTCGCATGTCATGCATGCCAGGGGGCGTCCGTGATTTTTACTG 660
Mnelk317c4           GCGGTTCCTTCCTGATTTTCGCATGTCATGCATGCCAGGGGGCGTCCGTGATTTTTACTG 660
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Mnelk317c3        GCGGTTCCTTCCTGATTTTCGCATGTCATGCATGCCAGGGGGCGTCCGTGATTTTTACTG 660
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AJ009164Tth       TGACTAAAAAAGTGTGACCAAAGCAGTCATTCGACTTGAATTAGAAAGCATGGGATAACA 720
Cow2073cl7        TGACTAAAAAAGTGTGACCAAAGCAGTCATTCGACTTGAATTAGAAAGCATGGGATAACA 719
AJ009163Tth       TGACTAAAAAAGTGTGACCAAAGCAGTCATTCGACTTGAATTAGAAAGCATGGGATAACA 720
Cow2073cl9        TGACTAAAAAAGTGTGACCAAAGCAGTCATTCGACTTGAATTAGAAAGCATGGGATAACA 720
AJ009165TspCd     TGACTAAAAAAGTGTGACCAAAGCAGTCATTCGACTTGAATTAGAAAGCATGGGATAACA 720
WTDA21cl6         TGACTAAAAAAGTGTGACCAAAGCAGTCATTCGACTTGAATTAGAAAGCATGGGATAACA 720
WTDA5cl1          TGACTAAAAAAGTGTGACCAAAGCAGTCATTCGACTTGAATTAGAAAGCATGGGATAACA 720
WTDA1Cl1          TGACTAAAAAAGTGTGACCAAAGCAGCCATTCGACTTGAATTAGAAAGCATGGGATAACA 720
MNelk328cl21      TGACTAAAAAAGTGTGACCAAAGCAGTCATTCAACTTGAATTAGAAAGCATGGGATAACA 720
WTD148cl4         TGACTAAAAAAGTGTGACCAAAGCAGTCATTCGACTTGAATTAGAAAGCATGGGATAACA 720
Cow138cl2         TGACTAAAAAAGTGTGACCAAAGCAGTCATTCGACTTGAATTAGAAAGCATGGGATAACA 720
WTD21cl4          TGACTAAAAAAGTGTGACCAAAGCAGTCATTCGACTTGAATTAGAAAGCATGGGATAACA 720
Cow138cl4         TGACTGAAAAAGTGTGACCAAAGCAGTCATTCGACTTGAATTAGAAAGCACGGGATAACA 720
WTDA1cl4          TGACTAAAAAAGTGTGACCAAAGCAGTCATTCGACTTGAATTAGAAAGCATGGGATAACA 720
WTDA21cl4         TGACTAAAAAAGTGTGACCAAAGCAGTCATTCGACTTGAATTAGAAAGCATGGGATAACA 720
MNelk328cl20      TGACTAAAAAAGTGTGACCAAAGCAGTCATTCGACTTGAATTAGAAAGCATGGGATAACA 720
Mnelk317c4        TGACTAAAAAAGTGTGACCAAAGCAGTCATTCGACTTGAATTAGAAAGCATGGGATAACA 720
Mnelk317c3        TGACTAAAAAAGTGTGACCAAAGCAGTCATTCGACTTGAATTAGAAAGCATGGGATAACA 720
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AJ009164Tth       AAGGAGCAGCCTATGGGCCACCGTTTCGGCTTTTGTTGGTTTTAAAAGTCCATTGGAGAT 780
Cow2073cl7        AAGGAGCAGCCTATGGGCCACCGTTTCGGCTTTTGTTGGTTTTAAAAGTCCATTGGAGAT 779
AJ009163Tth       AAGGAGCAGCCTATGGGCCACCGTTTCGGCTTTTGTTGGTTTTAAAAGTCCATTGGAGAT 780
Cow2073cl9        AAGGAGCAGCCTATGGGCCACCGTTTCGGCTTTTGTTGGTTTTAAAAGTCCATTGGAGAT 780
AJ009165TspCd     AAGGAGCAGCCTATGGGCCACCGTTTCGGCTTTTGTTGGTTTTAAAAGTCCATTGGAGAT 780
WTDA21cl6         AAGGAGCAGCCTATGGGCCACCGTTTCGGCTTTTGTTGGTTTTAAAAGTCCATTGGAGAT 780
WTDA5cl1          AAGGAGCAGCCTATGGGCCACCGTTTCGGCTTTTGTTGGTTTTAAAAGTCCATTGGAGAT 780
WTDA1Cl1          AAGGAGCAGCCTATGGGCCACCGTTTCGGCTTTTGTTGGTTTTAAAAGTCCATTGGAGAT 780
MNelk328cl21      AAGGAGCAGCCTATGGGCCACCGTTTCGGCTTTTGTTGGTTTTAAAAGTCCATTGGAGAT 780
WTD148cl4         AAGGAGCAGCCTATGGGCCACCGTTTCGGCTTTTGTTGGTTTTAAAAGTCCATTGGAGAT 780
Cow138cl2         AAGGAGCAGCCTATGGGCCACCGTTTCGGCTTTTGTTGGTTTTAAAAGTCCATTGGAGAT 780
WTD21cl4          AAGGAGCAGCCTATGGGCCACCGTTTCGGCTTTTGTTGGTTTTAAAAGTCCATTGGAGAT 780
Cow138cl4         AAGGAGCAGCCTATGGGCCACCGTTTCGGCTTTTGTTGGTTTTAAAAGTCCATTGGAGAT 780
WTDA1cl4          AAGGAGCAGCCTATGGGCCACCGTTTCGGCTTTTGTTGGTTTTAAAAGTCCATTGGAGAT 780
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WTDA21cl4          AAGGAGCAGCCTATGGGCCACCGTTTCGGCTTTTGTTGGTTTTAAAAGTCCATTGGAGAT 780
MNelk328cl20       AAGGAGCAGCCTATGGGCCACCGTTTCGGCTTTTGTTGGTTTTAAAAGTCCATTGGAGAT 780
Mnelk317c4         AAGGAGCAGCCTATGGGCCACCGTTTCGGCTTTTGTTGGTTTTAAAAGTCCATTGGAGAT 780
Mnelk317c3         AAGGAGCAGCCTATGGGCCACCGTTTCGGCTTTTGTTGGTTTTAAAAGTCCATTGGAGAT 780
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AJ009164Tth        TATGGCGTCGTGCGACAAGCGTCTGGGTGATTCCCC--TTTCGGGGCACCCGTCGCCTTT 838
Cow2073cl7         TATGGCGTCGTGCGACAAGCGTCTGGGTGATTCCCC--TTTCGGGGCACCCGTCGCCTTT 837
AJ009163Tth        TATGGCGTCGTGCGACAAGCGTCTGGGTGATTCCCC--TTTCGGGGCACCCGTCGCCTTT 838
Cow2073cl9         TATGGCGTCGTGCGACAAGCGTCTGGGTGATTCCCC--TTTCGGGGCACCCGTCGCCTTT 838
AJ009165TspCd      TATGGCGTCGTGCGACAAGCGTCTGGGTGATTCCCC--TTCTGGGGCACCCGTCGCCTTT 838
WTDA21cl6          TATGGCGTCGTGCGACAAGCGTCTGGGTGTTTTCCCATCTTTGGGGCACCCGTCGCCTTT 840
WTDA5cl1           TATGGCGTCGTGCGACAAGCGTCTGGGTGTTTTCCCAT-TTTGGGGCACCCGTCGCCTTT 839
WTDA1Cl1           TATGGCGTCGTGCGACAAGCGTCTGGGTGTTTTCCCAT-TTTGGGGCACCCGTCGCCTTT 839
MNelk328cl21       TATGGCGTCGTGCGACAAGCGTCTGGGTGTTTTCCCATTTTTGGGGCACCCGTCGCCTTT 840
WTD148cl4          TATGGCGTCGTGCGACAAGCGTCTGGGTGTTTTCCCATTTTTGGGGCACCCGTCGCCTTT 840
Cow138cl2          TATGGCGTCGTGCGACAAGCGTCTGGGTGTTTTCCCATCTTTGGGGCACCCGTCGCCTTT 840
WTD21cl4           TATGGCGTCGTGCGACAAGCGTCTGGGTGTTTTCCCATCTTTGGGGCACCCGTCGCCTTT 840
Cow138cl4          TATGGCGTCGTGCGACAAGCGTCTGGGTGTTTTCCCATCTTTGGGGCACCCGTCGCCTTT 840
WTDA1cl4           TATGGCGTCGTGCGACAAGCGTCTGGGTGTTTTCCCATCTTTGGGGCACCCGTCGCCTTT 840
WTDA21cl4          TATGGCGTCGTGCGACAAGCGTCTGGGTGTTTTCCCAT-TTTGGGGCACCCGTCGCCTTT 839
MNelk328cl20       TATGGCGTCGTGCGACAAGCGTCTGGGTGATTCCCC--TTTTGGGGCGCCCGTCGCCTTT 838
Mnelk317c4         TATGGCGTCGTGCGACAAGCGTCTGGGT-TTTCCTCATTTATGAGGTACCCGTCGCCTTT 839
Mnelk317c3         TATGGCGTCGTGCGACAAGCGTCTGGGT-TTTCCTCATTTATGAGGTACCCGTCGCCTTT 839
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AJ009164Tth        GCGGGAAATCCGTGTCGGTGTTG-ATGAGCTTCGGCCCATCTCTCCGGCGCCTTCCCGTG 897
Cow2073cl7         GCGGGAAATCCGTGTCGGTGTTG-ATGAGCTTCGGCCCATCTCTCCGGCGCCTTCCCGTG 896
AJ009163Tth        GCGGGAAATCCGTGTCGGTGTTG-ATGAGCTTCGGCCCATCTCTCCGGCGCCTTCCCGTG 897
Cow2073cl9         GCGGGAAATCCGTGTCGGTGTTG-ATGAGCTTCGGCCCATCTCTCCGGCGCCTTCCCGTG 897
AJ009165TspCd      GCGGGAAATCCGTGTCGGTGTTG-ATGAGCTTCGGCCCATCTCTCCGGCGCCTTCCCGTG 897
WTDA21cl6          GCGGGAAATCCGTGTCGGTGTTG-ATGAGCTTCGGCCCATCTCTCCGGCGCCTTCCCGTG 899
WTDA5cl1           GCGGGAAATCCGTGTCGGTGTTG-ATGAGCTTCGGCCCATCTCTCCGGCGCCTTCCCGTG 898
WTDA1Cl1           GCGGGAAATCCGTGTCGGTGTTG-ATGAGCTTCGGCCCATCTCTCCGGCGCCTTCCCGTG 898
MNelk328cl21       GCGGGAAATCCGTGTCGGTGTTG-ATGAGCTTCGGCCCATCTCTCCGGCGCCTTCCCGTG 899
WTD148cl4          GCGGGAAATCCGTGTCGGTGTTG-ATGAGCTTCGGCCCATCTCTCCGGCGCCTTCCCGTG 899
Cow138cl2          GCGAGAAATCCGTGTCGGTGTTG-ATGAGCTTCGGCCCATCTCTCCGGCGCCTTCTCGTG 899
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WTD21cl4          GCGAGAAATCCGTGTCGGTGTTG-ATGAGCTTCGGCCCATCTCTCCGGCGCCTTCTCGTG 899
Cow138cl4         GCGAGAAATCCGTGTCGGTGTTG-ATGAGCTTCGGCCCATCTCTCCGGCGCCTTCTCGTG 899
WTDA1cl4          GCGGGAAATCCGTGTCGGTGTTG-ATGAGCTTCGGCCCATCTCTCCGGCGCCTTCCCGTG 899
WTDA21cl4         GCGGGAAATCCGTGTCGGTGTTG-ATGAGCTTCGGCCCATCTCTCCGGCGCCTTCCCGTG 898
MNelk328cl20      GCGGGAAATCCGTGTCGGTGTTG-ATGAGCTTCGGCCCATCTCTCCGGCGCCTTCCCGTG 897
Mnelk317c4        GCGGGAAATCCGTGTCGGTGCTGTATGGGTTTCGGCCCATCTGCCCGGCGCCTTCCCGTG 899
Mnelk317c3        GCGGGAAATCCGTGTCGGTGCTGTATGGGTTTCGGCCCATCTGCCCGGCGCCTTCCCGTG 899
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AJ009164Tth       ACTCACGGCATCCAGGAATGAAGGAGGGTAGTTCGGGGGAGAACGTACTGGCGCGTCAGA 957
Cow2073cl7        ACTCACGGCATCCAGGAATGAAGGAGGGTAGTTCGGGGGAGAACGTACTGGCGCGTCAGA 956
AJ009163Tth       ACTCACGGCATCCAGGAATGAAGGAGGGTAGTTCGGGGGAGAACGTACTGGCGCGTCAGA 957
Cow2073cl9        ACTCACGGCATCCAGGAATGAAGGAGGGTAGTTCGGGGGAGAACGTACTGGCGCGTCAGA 957
AJ009165TspCd     ACTCACGGCATCCAGGAATGAAGGAGGGTAGTTCGGGGGAGAACGTACTGGCGCGTCAGA 957
WTDA21cl6         ACTCACGGCATCCAGGAATGAAGGAGGGTAGTTCGGGGGAGAACGTACTGGCGCGTCAGA 959
WTDA5cl1          ACTCACGGCATCCAGGAATGAAGGAGGGTAGTTCGGGGGAGAACGTACTGGCGCGTCAGA 958
WTDA1Cl1          ACTCACGGCATCCAGGAATGAAGGAGGGTAGTTCGGGGGAGAACGTACTGGCGCGTCAGA 958
MNelk328cl21      ACTCACGGCATCCAGGAATGAAGGAGGGTAGTTCGGGGGAGAACGTACTGGCGCGTCAGA 959
WTD148cl4         ACTCACGGCATCCAGGAATGAAGGAGGGTAGTTCGGGGGAGAACGTACTGGCGCGTCAGA 959
Cow138cl2         ACTCACGGCATCCAGGAATGAAGGAGGGTAGTTCGGGGGAGAACGTACTGGCGCGTCAGA 959
WTD21cl4          ACTCACGGCATCCAGGAATGAAGGAGGGTAGTTCGGGGGAGAACGTACTGGCGCGTCAGA 959
Cow138cl4         ACTCACGGCATCCAGGAATGAAGGAGGGTAGTTCGGGGGAGAACGTACTGGCGCGTCAGA 959
WTDA1cl4          ACTCACGGCATCCAGGAATGAAGGAGGGTAGTTCGGGGGAGAACGTACTGGCGCGTCAGA 959
WTDA21cl4         ACTCACGGCATCCAGGAATGAAGGAGGGTAGTTCGGGGGGGAACGTACTGGCGCGTCAGA 958
MNelk328cl20      ACTCACGGCATCCAGGAATGAAGGAGGGTAGTTCGGGGGAGAACGTACTGGCGCGTCAGA 957
Mnelk317c4        ACTCACGGCATCCAGGAATGAAGGAGGGTAGTTCGGGGGAGAACGTACTGGCGCGTCAGA 959
Mnelk317c3        ACTCACGGCATCCAGGAATGAAGGAGGGTAGTTCGGGGGAGAACGTACTGGCGCGTCAGA 959
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AJ009164Tth       GGTGAAATTCTTAGACCGCGCCAAGACGAACTACAGCGAAGGCATTCTTCAAGGATACCT 1017
Cow2073cl7        GGTGAAATTCTTAGACCGCGCCAAGACGAACTACAGCGAAGGCATTCTTCAAGGATACCT 1016
AJ009163Tth       GGTGAAATTCTTAGACCGCGCCAAGACGAACTACAGCGAAGGCATTCTTCAAGGATACCT 1017
Cow2073cl9        GGTGAAATTCTTAGACCGCGCCAAGACGAACTACAGCGAAGGCATTCTTCAAGGATACCT 1017
AJ009165TspCd     GGTGAAATTCTTAGACCGCGCCAAGACGAACTACAGCGAAGGCATTCTTCAAGGATACCT 1017
WTDA21cl6         GGTGAAATTCTTAGACCGCGCCAAGACGAACTACAGCGAAGGCATTCTTCAAGGATACCT 1019
WTDA5cl1          GGTGAAATTCTTAGACCGCGCCAAGACGAACTACAGCGAAGGCATTCTTCAAGGATACCT 1018
WTDA1Cl1          GGTGAAATTCTTAGACCGCGCCAAGACGAACTACAGCGAAGGCATTCTTCAAGGATACCT 1018
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MNelk328cl21      GGTGAAATTCTTAGACCGCGCCAAGACGAACTACAGCGAAGGCATTCTTCAAGGATACCT 1019
WTD148cl4         GGTGAAATTCTTAGACCGCGCCAAGACGAACTACAGCGAAGGCATTCTTCAAGGATACCT 1019
Cow138cl2         GGTGAAATTCTTAGACCGCGCCAAGACGAACTACAGCGAAGGCATTCTTCAAGGATACCT 1019
WTD21cl4          GGTGAAATTCTTAGACCGCGCCAAGACGAACTACAGCGAAGGCATTCTTCAAGGATACCT 1019
Cow138cl4         GGTGAAATTCTTAGACCGTGCCAAGACGAACTACAGCGAAGGCATTCTTCAAGGATACCT 1019
WTDA1cl4          GGTGAAATTCTTAGACCGCGCCAAGACGAACTACAGCGAAGGCATTCTTCAAGGATACCT 1019
WTDA21cl4         GGTGAAATTCTTAGACCGCGCCAAGACGAACTACAGCGAAGGCATTCTTCAAGGATACCT 1018
MNelk328cl20      GGTGAAATTCTTAGACCGCGCCAAGACGAACTACAGCGAAGGCATTCTTCAAGGATACCT 1017
Mnelk317c4        GGTGAAATTCTTAGACCGCGCCAAGACGAACTACAGCGAAGGCATTCTTCAAGGATACCT 1019
Mnelk317c3        GGTGAAATTCTTAGACCGCGCCAAGACGAACTACAGCGAAGGCATTCTTCAAGGATACCT 1019
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AJ009164Tth       TCCTCAATCAAGAACCAAAGTGTGGGGATCGAAGATGATTAGAGACCATTGTAGTCCACA 1077
Cow2073cl7        TCCTCAATCAAGAACCAAAGTGTGGGGATCGAAGATGATTAGAGACCATTGTAGTCCACA 1076
AJ009163Tth       TCCTCAATCAAGAACCAAAGTGTGGGGATCGAAGATGATTAGAGACCATTGTAGTCCACA 1077
Cow2073cl9        TCCTCAATCAAGAACCAAAGTGTGGGGATCGAAGATGATTAGAGACCATTGTAGTCCACA 1077
AJ009165TspCd     TCCTCAATCAAGAACCAAAGTGTGGGGATCGAAGATGATTAGAGACCATTGTAGTCCACA 1077
WTDA21cl6         TCCTCAATCAAGAACCAAAGTGTGGGGATCGAAGATGATTAGAGACCATTGTAGTCCACA 1079
WTDA5cl1          TCCTCAATCAAGAACCAAAGTGTGGGGATCGAAGATGATTAGAGACCATTGTAGTCCACA 1078
WTDA1Cl1          TCCTCAATCAAGAACCAAAGTGTGGGGATCGAAGATGATTAGAGACCATTGTAGTCCACA 1078
MNelk328cl21      TCCTCAATCAAGAACCAAAGTGTGGGGATCGAAGATGATTAGAGACCATTGTAGTCCACA 1079
WTD148cl4         TCCTCAATCAAGAACCAAAGTGTGGGGATCGAAGATGATTAGAGACCATTGTAGTCCACA 1079
Cow138cl2         TCCTCAATCAAGAACCAAAGTGTGGGGATCGAAGATGATTAGAGACCATTGTAGTCCACA 1079
WTD21cl4          TCCTCAATCAAGAACCAAAGTGTGGGGATCGAAGATGATTAGAGACCATTGTAGTCCACA 1079
Cow138cl4         TCCTCAATCAAGAACCAAAGTGTGGGGGTCGAAGATGATTAGAGACCATTGTAGTCCACA 1079
WTDA1cl4          TCCTCAATCAAGAACCAAAGTGTGGGGATCGAAGATGATTAGAGACCATTGTAGTCCACA 1079
WTDA21cl4         TCCTCAATCAAGAACCAAAGTGTGGGGATCGAAGATGATTAGAGACCATTGTAGTCCACA 1078
MNelk328cl20      TCCTCAATCAAGAACCAAAGTGTGGGGATCGAAGATGATTAGAGACCATTGTAGTCCACA 1077
Mnelk317c4        TCCTCAATCAAGAACCAAAGTGTGGGGATCGAAGATGATTAGAGACCATTGTAGTCCACA 1079
Mnelk317c3        TCCTCAATCAAGAACCAAAGTGTGGGGATCGAAGATGATTAGAGACCATTGTAGTCCACA 1079
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AJ009164Tth       CTGCAAACGATGACACCCATGAATTGGGGAATTTTTGGTCGCAGGCGGGGTCGAGTTCAT 1137
Cow2073cl7        CTGCAAACGATGACACCCATGAATTGGGGAATTTTTGGTCGCAGGCGGGGTCGAGTTCAT 1136
AJ009163Tth       CTGCAAACGATGACACCCATGAATTGGGGAATTTTTGGTCGCAGGCGGGGTCGAGTTCAT 1137
Cow2073cl9        CTGCAAACGATGACACCCATGAATTGGGGAATTTTTGGTCGCAGGCGGGGTCGAGTTCAT 1137
AJ009165TspCd     CTGCAAACGATGACACCCATGAATTGGGGAATTTTTGGTCGCAGGCGGGGTCGAGTTCAT 1137
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WTDA21cl6         CTGCAAACGATGACACCCATGAATTGGGGAATTTTTGGTCGCAGGCGGGGTCGAGTTCAT 1139
WTDA5cl1          CTGCAAACGATGACACCCATGAATTGGGGAATTTTTGGTCGTAGGCGAGGTCGGGTTCAT 1138
WTDA1Cl1          CTGCAAACGATGACACCCATGAATTGGGGAATTTTTGGTCGTAGGCGAGGTCGGGTTCAT 1138
MNelk328cl21      CTGCAAACGATGACACCCATGAATTGGGGAATTTTTGGTCGTAGGCGAGGTCGGGTTCAT 1139
WTD148cl4         CTGCAAACGATGACACCCATGAATTGGGGAATTTTTGGTCGTAGGCGAGGTCGGGTTCAT 1139
Cow138cl2         CTGCAAACGATGACACCCATGAATTGGGGAATTTTTGGTCGTAGGCGAGGCCGGGTTCAT 1139
WTD21cl4          CTGCAAACGATGACACCCATGAATTGGGGAATTTTTGGTCGTAGGCGAGGCCGGGTTCAT 1139
Cow138cl4         CTGCAAACGATGACACCCATGAATTGGGGAATTTTTGGTCGTAGGCGAGGTCGGGTTCAT 1139
WTDA1cl4          CTGCAAACGATGACACCCATGAATTGGGGAATTTTTGGTCGTAGGCGAGGTCGGGTTCAT 1139
WTDA21cl4         CTGCAAACGATGACACCCATGAATTGGGGAATTTTTGGTCGTAGGCGAGGTCGGGTTCAT 1138
MNelk328cl20      CTGCAAACGATGACACCCATGAATTGGGGAATTTTTGGTCGTAGGCGAGGTCGGGTTCAT 1137
Mnelk317c4        CTGCAAACGATGACACCCATGAATTGGGGAATTTTTGGTCGCAGGCGGGGTCGGGTTCAT 1139
Mnelk317c3        CTGCAAACGATGACACCCATGAATTGGGGAATTTTTGGTCGCAGGCGGGGTCGGGTTCAT 1139
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AJ009164Tth       CTCGCTCCTCGCCTC 1152
Cow2073cl7        CTCGCTCCTCGCCTC 1151
AJ009163Tth       CTCGCTCCTCGCCTC 1152
Cow2073cl9        CTCGCTCCTCGCCTC 1152
AJ009165TspCd     CTCGCTCCTCGCCTC 1152
WTDA21cl6         CTCGCTCCTCGCCTC 1154
WTDA5cl1          CTCGCTCCTCGCCTC 1153
WTDA1Cl1          CTCGCTCCTCGCCTC 1153
MNelk328cl21      CTCGCTCCTCGCCTC 1154
WTD148cl4         CTCGCTCCTCGCCTC 1154
Cow138cl2         CTCGCTCCTCGCCTC 1154
WTD21cl4          CTCGCTCCTCGCCTC 1154
Cow138cl4         CTCGCTCCTCGCCTC 1154
WTDA1cl4          CTCGCTCCTCGCCTC 1154
WTDA21cl4         CTCGCTCCTCGCCTC 1153
MNelk328cl20      CTCGCTCCTCGCCTC 1152
Mnelk317c4        CTCGCTCCTCGCCTC 1154
Mnelk317c3        CTCGCTCCTCGCCTC 1154
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# APPENDIX B

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CLUSTALW of 1500 bp of 18S rRNA, ITS1-5.8S-ITS2 rDNA gene

Elk142c2          CACCCGCGGTAATTCCAGCTCCAAAAGCGTATATTAATGCTGTTGCTGTTAAAGGGTTCG 60
Elk142c10         CACCCGCGGTAATTCCAGCTCCAAAAGCGTATATTAATGCTGTTGCTGTTAAAGGGTTCG 60
Elk142c5          CACCCGCGGTAATTCCAGCTCCAAAAGCGTATATTAATGCTGTTGCTGTTAAAGGGTTCG 60
Elk328c3          CACCCGCGGTAATTCCAGCTCCAAAAGCGTATATTAATGCTGTTGCTGTTAAAGGGTTCG 60
Elk416c8          CACCCGCGGTAATTCCAGCTCCAAAAGCGTATATTAATGCTGTTGCTGTTAAAGGGTTCG 60
WTDN15c1          CACCCGCGGTAATTCCAGCTCCAAAAGCGTATATTAATGCTGTTGCTGTTAAAGGGTTCG 60
WTDNL15c6         CACCCGCGGTAATTCCAGCTCCAAAAGCGTATATTAATGCTGTTGCTGTTAAAGGGTTCG 60
WTDN15c9          CACCCGCGGTAATTCCAGCTCCAAAAGCGTATATTAATGCTGTTGCTGTTAAAGGGTTCG 60
WTDA21c4          CACCCGCGGTAATTCCAGCTCCAAAAGCGTATATTAATGCTGTTGCTGTTAAAGGGTTCG 60
WTDA21c5          CACCCGCGGTAATTCCAGCTCCAAAAGCGTATATTAATGCTGTTGCTGTTAAAGGGTTCG 60
WTDA21c6          CACCCGCGGTAATTCCAGCTCCAAAAGCGTATATTAATGCTGTTGCTGTTAAAGGGTTCG 60
Cow139c10         CACCCGCGGTAATTCCAGCTCCAAAAGCGTATATTAATGCTGTTGCTGTTAAAGGGTTCG 60
Cow139c11         CACCCGCGGTAATTCCAGCTCCAAAAGCGTATATTAATGCTGTTGCTGTTAAAGGGTTCG 60
Cow2c4            CACCCGCGGTAATTCCAGCTCCAAAAGCGTATATTAATGCTGTTGCTGTTAAAGGGTTCG 60
Cow133c1          CACCCGCGGTAATTCCAGCTCCAAAAGCGTATATTAATGCTGTTGCTGTTAAAGGGTTCG 60
Cow139c9          CACCCGCGGTAATTCCAGCTCCAAAAGCGTATATTAATGCTGTTGCTGTTAAAGGGTTCG 60
Cow3535c3         CACCCGCGGTAATTCCAGCTCCAAAAGCGTATATTAATGCTGTTGCTGTTAAAGGGTTCG 60
Cow3535c6         CACCCGCGGTAATTCCAGCTCCAAAAGCGTATATTAATGCTGTTGCTGTTAAAGGGTTCG 60
Cow2c7            CACCCGCGGTAATTCCAGCTCCAAAAGCGTATATTAATGCTGTTGCTGTTAAAGGGTTCG 60
Cow2c10           CACCCGCGGTAATTCCAGCTCCAAAAGCGTATATTAATGCTGTTGCTGTTAAAGGGTTCG 60
AB007814Tth       CACCCGCGGTAATTCCAGCTCCAAAAGCGTATATTAATGCTGTTGCTGTTAAAGGGTTCG 60
Cow104c2          CACCCGCGGTAATTCCAGCTCCAAAAGCGTATATTAATGCTGTTGCTGTTAAAGGGTTCG 60
Cow104c3          CACCCGCGGTAATTCCAGCTCCAAAAGCGTATATTAATGCTGTTGCTGTTAAAGGGTTCG 60
Cow104c1          CACCCGCGGTAATTCCAGCTCCAAAAGCGTATATTAATGCTGTTGCTGTTAAAGGGTTCG 60
Cow2095c4         CACCCGCGGTAATTCCAGCTCCAAAAGCGTATATTAATGCTGTTGCTGTTAAAGGGTTCG 60
Cow2095c9         CACCCGCGGTAATTCCAGCTCCAAAAGCGTATATTAATGCTGTTGCTGTTAAAGGGTTCG 60
Cow2095c8         CACCCGCGGTAATTCCAGCTCCAAAAGCGTATATTAATGCTGTTGCTGTTAAAGGGTTCG 60
WTDA3c9.1         CACCCGCGGTAATTCCAGCTCCAAAAGCGTATATTAATGCTGTTGCTGTTAAAGGGTTCG 60
WTDA3c9.4         CACCCGCGGTAATTCCAGCTCCAAAAGCGTATATTAATGCTGTTGCTGTTAAAGGGTTCG 60
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Elk142c2          TAGTTGAATTGTGGGCCTTTGAGGCGCAATGGTTTGTCCCGTCCACCTCGGATTGGTGAC 120
Elk142c10         TAGTTGAATTGTGGGCCTTTGAGGCGCAATGGTTTGTCCCGTCCACCTCGGATTGGTGAC 120
Elk142c5          TAGTTGAATTGTGGGCCTTTGAGGCGCAATGGTTTGTCCCGTCCACCTCGGATTGGTGAC 120
Elk328c3          TAGTTGAATTGTGGGCCTTTGAGGCGCAATGGTTCGTCCCGTCCACCTCGGATTGGTGAC 120
Elk416c8          TAGTTGAATTGTGGGCCTTTGAGGCGCAATGGTTTGTCCCGTCCACCTCGGATTGGTGAC 120
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WTDN15c1        TAGTTGAATTGTGGGCCTTTGAGGCGCAATGGTTTGTCCCGTCCACCTCGGATTGGTGAC 120
WTDNL15c6       TAGTTGAATTGTGGGCCTTTGAGGCGCAATGGTTTGTCCCGTCCACCTCGGATTGGTGAC 120
WTDN15c9        TAGTTGAATTGTGGGCCTTTGAGGCGCAATGGTTTGTCCCGTCCACCTCGGATTGGTGAC 120
WTDA21c4        TAGTTGAATTGTGGGCCTTTGAGGCGCAATGGTTTGTCCCGTCCACCTCGGATTGGTGAC 120
WTDA21c5        TAGTTGAATTGTGGGCCTTTGAGGCGCAATGGTTTGTCCCGTCCACCTCGGATTGGTGAC 120
WTDA21c6        TAGTTGAATTGTGGGCCTTTGAGGCGCAATGGTTTGTCCCGTCCACCTCGGATTGGTGAC 120
Cow139c10       TAGTTGAATTGTGGGCCTTTGAGGCGCAATGGTTTGTCCCGTCCACCTCGGATTGGTGAC 120
Cow139c11       TAGTTGAATTGTGGGCCTTTGAGGCGCAATGGTTTGTCCCGTCCACCTCGGATTGGTGAC 120
Cow2c4          TAGTTGAATTGTGGGCCTTTGAGGCGCAATGGTTTGTCCCGTCCACCTCGGATTGGTGAC 120
Cow133c1        TAGTTGAATTGTGGGCCTTTGAGGCGCAATGGTTTGTCCCGTCCACCTCGGATTGGTGAC 120
Cow139c9        TAGTTGAATTGTGGGCCTTTGAGGCGCAGTGGTTTGTCCCGTCCACCTCGGATTGGTGAC 120
Cow3535c3       TAGTTGAATTGTGGGCCTTTGAGGCGCAATGGTTTGTCCCGTCCACCTCGGATTGGTGAC 120
Cow3535c6       TAGTTGAATTGTGGGCCTTTGAGGCGCAATGGTTTGTCCCGTCCACCGCGGATTGGTGAC 120
Cow2c7          TAGTTGAATTGTGGGCCTTTGAGGCGCAATGGTTTGTCCCGTCCACCTCGGATTGGTGAC 120
Cow2c10         TAGTTGAATTGTGGGCCTTTGAGGCGCAATGGTTTGTCCCGTCCACCTCGGATTGGTGAC 120
AB007814Tth     TAGTTGAATTGTGGGCCTTTGAGGCGCAATGGTTTGTCCCGTCCACCTCGGATTGGTGAC 120
Cow104c2        TAGTTGAATTGTGGGCCTTTGAGGCGCAATGGTTTGTCCCGTCCACCTCGGATTGGTGAC 120
Cow104c3        TAGTTGAATTGTGGGCCTTTGAGGCGCAATGGTTTGTCCCGTCCACCTCGGATTGGTGAC 120
Cow104c1        TAGTTGAATTGTGGGCCTTTGAGGCGCAATGGTTTGTCCCGTCCACCTCGGATTGGTGAC 120
Cow2095c4       TAGTTGAATTGTGGGCCTTTGAGGCGCAATGGTTTGTCCCGTCCACCTCGGATTGGTGAC 120
Cow2095c9       TAGTTGAATTGTGGGCCTTTGAGGCGCAATGGTTTGTCCCGTCCACCTCGGATTGGTGAC 120
Cow2095c8       TAGTTGAATTGTGGGCCTTTGAGGCGCAATGGTTTGTCCCGTCCACCTCGGATTGGTGAC 120
WTDA3c9.1       TAGTTGAATTGTGGGCCTTTGAGGCGCAATGGCTTGTCCCGTCCACCTCGGATTGGTGAC 120
WTDA3c9.4       TAGTTGAATTGTGGGCCTTTGAGGCGCAATGGCTTGTCCCGTCCACCTCGGATTGGTGAC 120
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Elk142c2        CCATGCCCTTGAGGTCCGTGAACACTCAGAAACAAGAAACACGGGAGCGGTTCCTTCCTG 180
Elk142c10       CCATGCCCTTGAGGTCCGTGAACACTCAGAAACAAGAAACACGGGAGCGGTTCCTTCCTG 180
Elk142c5        CCATGCCCTTGAGGTCCGTGAACACTCAGAAACAAGAAACACGGGAGCGGTTCCTTCCTG 180
Elk328c3        CCATGCCCTTGAGGTCCGTGAACACTCAGAAACAAGAAACACGGGAGCGGTTCCTTCCTG 180
Elk416c8        CCATGCCCTTGAGGTCCGTGAACACTCAGAAACAAGAAACACGGGAGCGGTTCCTTCCTG 180
WTDN15c1        CCATGCCCTTGAGGTCCGTGAACACTCAGAAACAAGAAACACGGGAGCGGTTCCTTCCTG 180
WTDNL15c6       CCATGCCCTTGAGGTCCGTGAACACTCAGAAACAAGAAACACGGGAGCGGTTCCTTCCTG 180
WTDN15c9        CCATGCCCTTGAGGTCCGTGAACACTCAGAAACAAGAAACACGGGAGCGGTTCCTTCCTG 180
WTDA21c4        CCATGCCCTTGAGGTCCGTGAACACTCAGAAACAAGAAACACGGGAGCGGTTCCTTCCTG 180
WTDA21c5        CCATGCCCTTGAGGTCCGTGAACACTCAGAAACAAGAAACACGGGAGCGGTTCCTTCCTG 180
WTDA21c6        CCATGCCCTTGAGGTCCGTGAACACTCAGAAACAAGAAACACGGGAGCGGTTCCTTCCTG 180
Cow139c10       CCATGCCCTTGAGGTCCGTGAACAATCAGAAACAAAAAACACGGGAGCGGTTCCTTCCTG 180
Cow139c11       CCATGCCCTTGAGGTCCGTGAACAATCAGAAACAAAAAACACGGGAGCGGTTCCTTCCTG 180
Cow2c4          CCATGCCCTTGAGGTCCGTGAACAATCAGAAACAAAAAACACGGGAGCGGTTCCTTCCTG 180
Cow133c1        CCATGCCCTTGAGGTCCGTGAACAATCAGAAACAAAAAACACGGGAGCGGTTCCTTCCTG 180
Cow139c9        CCATGCCCTTGAGGTCCGTGAACAATCAGAAACAAAAAACACGGGAGCGGTTCCTTCCTG 180
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Cow3535c3        CCATGCCCTTGAGGTCCGTGAACAATCAGAAACAAAAAACACGGGAGCGGTTCCTTCCTG 180
Cow3535c6        CCATGCCCTTGAGGTCCGTGAACAATCAGAAACAAAAAACACGGGAGCGGTTCCTTCCTG 180
Cow2c7           CCATGCCCTTGAGGTCCGTGAACAATCAGAAACAAAAAACACGGGAGCGGTTCCTTCCTG 180
Cow2c10          CCATGCCCTTGAGGTCCGTGAACAATCAGAAACAAAAAACACGGGAGCGGTTCCTTCCTG 180
AB007814Tth      CCATGCCCTTGAGGTCCGTGAACAATCAGAAACAAAAAACACGGGAGCGGTTCCTTCCTG 180
Cow104c2         CCATGCCCTTGAGGTCCGTGAACAATCAGAAACAAAAAACACGGGAGCGGTTCCTTCCTG 180
Cow104c3         CCATGCCCTTGAGGTCCGTGAACAATCAGAAACAAAAAACACGGGAGCGGTTCCTTCCTG 180
Cow104c1         CCATGCCCTTGAGGTCCGTGAATAATCAGAAACAAAAAACACGGGAGCGGTTCCTTCCTG 180
Cow2095c4        CCATGCCCTTGAGGTCCGTGAACAATCAGAAACAAAAAACACGGGAGCGGTTCCTTCCTG 180
Cow2095c9        CCATGCCCTTGAGGTCCGTGAACAATCAGAAACAAAAAACACGGGAGCGGTTCCTTCCTG 180
Cow2095c8        CCATGCCCTTGAGGTCCGTGAACAATCAGAAACAAAAAACACGGGAGCGGTTCCTTCCTG 180
WTDA3c9.1        CCATGCCCTTGAGGTCCGTGAACACTCAGAAACAAGAAACACGGGAGCGGTTCCTTCCTG 180
WTDA3c9.4        CCATGCCCTTGAGGTCCGTGAACACTCAGAAACAAGAAACACGGGAGCGGTTCCTTCCTG 180
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Elk142c2         ATTTTCGCATGTCATGCATGCCAGGGGGCGTCCGTGATTTTTACTGTGACTAAAAAAGTG 240
Elk142c10        ATTTTCGCATGTCATGCATGCCAGGGGGCGTCCGTGATTTTTACTGTGACTAAAAAAGTG 240
Elk142c5         ATTTTCGCATGTCATGCATGCCAGGGGGCGTCCGTGATTTTTACTGTGACTAAAAAAGTG 240
Elk328c3         ATTTTCGCATGTCATGCATGCCAGGGGGCGTCCGTGATTTTTACTGTGACTAAAAAAGTG 240
Elk416c8         ATTTTCGCATGTCATGCATGCCAGGGGGCGTCCGTGATTTTTACTGTGACTAAAAAAGTG 240
WTDN15c1         ATTTTCGCATGTCATGCATGCCAGGGGGCGTCCGTGATTTTTACTGTGACTAAAAAAGTG 240
WTDNL15c6        ATTTTCGCATGTCATGCATGCCAGGGGGCGTCCGTGATTTTTACTGTGACTAAAAAAGTG 240
WTDN15c9         ATTTTCGCATGTCATGCATGCCAGGGGGCGTCCGTGATTTTTACTGTGACTAAAAAAGTG 240
WTDA21c4         ATTTTCGCATGTCATGCATGCCAGGGGGCGTCCGTGATTTTTACTGTGACTAAAAAAGTG 240
WTDA21c5         ATTTTCGCATGTCATGCATGCCAGGGGGCGTCCGTGATTTTTACTGTGACTAAAAAAGTG 240
WTDA21c6         ATTTTCGCATGTCATGCATGCCAGGGGGCGTCCGTGATTTTTACTGTGACTAAAAAAGTG 240
Cow139c10        ATTTTCGCATGTCATGCATGCCAGGGGGCGTCCGTGATTTTTACTGTGACTAAAAAAGTG 240
Cow139c11        ATTTTCGCATGTCATGCATGCCAGGGGGCGTCCGTGATTTTTACTGTGACTAAAAAAGTG 240
Cow2c4           ATTTTCGCATGTCATGCATGCCAGGGGGCGTCCGTGATTTTTACTGTGACTAAAAAAGTG 240
Cow133c1         ATTTTCGCATGTCATGCATGCCAGGGGGCGTCCGTGATTTTTACTGTGACTAAAAAAGTG 240
Cow139c9         ATTTTCGCATGTCATGCATGCCAGGGGGCGTCCGTGATTTTTACTGTGACTAAAAAAGTG 240
Cow3535c3        ATTTTCGCATGTCATGCATGCCAGGGGGCGTCCGTGATTTTTACTGTGACTAAAGAAGTG 240
Cow3535c6        ATTTTCGCATGTCATGCATGCCAGGGGGCGTCCGTGATTTTTACTGTGACTAAAAAAGTG 240
Cow2c7           ATTTTCGCATGTCATGCATGCCAGGGGGCGTCCGTGATTTTTACTGTGACTAAAAAAGTG 240
Cow2c10          ATTTTCGCATGTCATGCATGCCAGGGGGCGTCCGTGATTTTTACTGTGACTAAAAAAGTG 240
AB007814Tth      ATTTTCGCATGTCATGCATGCCAGGGGGCGTCCGTGATTTTTACTGTGACTAAAAAAGTG 240
Cow104c2         ATTTTCGCATGTCATGCATGCCAGGGGGCGTCCGTGATTTTTACTGTGACTAAAAAAGTG 240
Cow104c3         ATTTTCGCATGTCATGCATGCCAGGGGGCGTCCGTGATTTTTACTGTGACTAAAAAAGTG 240
Cow104c1         ATTTTCGCATGTCATGCATGCCAGGGGGCGTCCGTGATTTTTACTGTGACTAAAAAAGTG 240
Cow2095c4        ATTTTCGCATGTCATGCATGCCAGGGGGCGTCCGTGATTTTTACTGTGACTAAAAAAGTG 240
Cow2095c9        ATTTTCGCATGTCATGCATGCCAGGGGGCGTCCGTGATTTTTACTGTGACTAAAAAAGTG 240
Cow2095c8        ATTTTCGCATGTCATGCATGCCAGGGGGCGTCCGTGATTTTTACTGTGACTAAAAAAGTG 240
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WTDA3c9.1        ATTTTCGCATGTCATGCATGCCAGGGGGCGTCCGTGATTTTTACTGTGACTAAAAAAGTG 240
WTDA3c9.4        ATTTTCGCATGTCATGCATGCCAGGGGGCGTCCGTGATTTTTACTGTGACTAAAAAAGTG 240
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Elk142c2         TGACCAAAGCAGTCATTCGACTTGAATTAGAAAGCATGGGATAACAAAGGAGCAGCCTAT 300
Elk142c10        TGACCAAAGCAGTCATTCGACTTGAATTAGAAAGCATGGGATAACAAAGGAGCAGCCTAT 300
Elk142c5         TGACCAAAGCAGTCATTCGACTTGAATTAGAAAGCATGGGATAACAAAGGAGCAGCCTAT 300
Elk328c3         TGACCAAAGCAGTCATTCGACTTGAATTAGAAAGCATGGGATAACAAAGGAGCAGCCTAT 300
Elk416c8         TGACCAAAGCAGTCATTCGACTTGAATTAGAAAGCATGGGATAACAAAGGAGCAGCCTAT 300
WTDN15c1         TGACCAAAGCAGTCATTCGACTTGAATTAGAAAGCATGGGATAACAAAGGAGCAGCCTAT 300
WTDNL15c6        TGACCAAAGCAGTCATTCGACTTGAATTAGAAAGCATGGGATAACAAAGGAGCAGCCTAT 300
WTDN15c9         TGACCAAAGCAGTCATTCGACTTGAATTAGAAAGCATGGGATAACAAAGGAGCAGCCTAT 300
WTDA21c4         TGACCAAAGCAGTCATTCGACTTGAATTAGAAAGCATGGGATAACAAAGGAGCAGCCTAT 300
WTDA21c5         TGACCAAAGCAGTCATTCGACTTGAATTAGAAAGCATGGGATAACAAAGGAGCAGCCTAT 300
WTDA21c6         TGACCAAAGCAGTCATTCGACTTGAATTAGAAAGCATGGGATAACAAAGGAGCAGCCTAT 300
Cow139c10        TGACCAAAGCAGTCATTCGACTTGAATTAGAAAGCATGGGATAACAAAGGAGCAGCCTAT 300
Cow139c11        TGACCAAAGCAGTCATTCGACTTGAATTAGAAAGCATGGGATAACAAAGGAGCAGCCTAT 300
Cow2c4           TGACCAAAGCAGTCATTCGACTTGAATTAGAAAGCATGGGATAACAAAGGAGCAGCCTAT 300
Cow133c1         TGACCAAAGCAGTCATTCGACTTGAATTAGAAAGCATGGGATAACAAAGGAGCAGCCTAT 300
Cow139c9         TGACCAAAGCAGTCATTCGACTTGAATTAGAAAGCATGGGATAACAAAGGAGCAGCCTAT 300
Cow3535c3        TGACCAAAGCAGTCATTCGACTTGAATTAGAAAGCATGGGATAACAAAGGAGCAGCCTAT 300
Cow3535c6        TGACCAAAGCAGTCATTCGACTTGAATTAGAAAGCATGGGATAACAAAGGAGCAGCCTAT 300
Cow2c7           TGACCAAAGCAGTCATTCGACTTGAATTAGAAAGCATGGGATAACAAAGGAGCAGCCTAT 300
Cow2c10          TGACCAAAGCAGTCATTCGACTTGAATTAGAAAGCATGGGATAACAAAGGAGCAGCCTAT 300
AB007814Tth      TGACCAAAGCAGTCATTCGACTTGAATTAGAAAGCATGGGATAACAAAGGAGCAGCCTAT 300
Cow104c2         TGACCAAAGCAGTCATTCGACTTGAATTAGAAAGCATGGGATAACAAAGGAGCAGCCTAT 300
Cow104c3         TGACCAAAGCAGTCATTCGACTTGAATTAGAAAGCATGGGATAACAAAGGAGCAGCCTAT 300
Cow104c1         TGACCAAAGCAGTCATTCGACTTGAATTAGAAAGCATGGGATAACAAAGGAGCAGCCTAT 300
Cow2095c4        TGACCAAAGCAGTCATTCGACTTGAATTAGAAAGCATGGGATAACAAAGGAGCAGCCTAT 300
Cow2095c9        TGACCAAAGCAGTCATTCGACTTGAATTAGAAAGCATGGGATAACAAAGGAGCAGCCTAT 300
Cow2095c8        TGACCAAAGCAGTCATTCGACTTGAATTAGAAAGCATGGGATAACAAAGGAGCAGCCTAT 300
WTDA3c9.1        TGACCAAAGCAGTCATTCGACTTGAATTAGAAAGCATGGGATAACAAAGGAGCAGCCTAT 300
WTDA3c9.4        TGACCAAAGCAGTCATTCGACTTGAATTAGAAAGCATGGGATAACAAAGGAGCAGCCTAT 300
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Elk142c2         GGGCCACCGTTTTGGCTTTTGTTGGTTTTAAAAGTCCATTGGAGATTATGGCGTCGTGCG 360
Elk142c10        GGGCCACCGTTTCGGCTTTTGTTGGTTTTAAAAGTCCATTGGAGATTATGGCGTCGTGCG 360
Elk142c5         GGGCCACCGTTTCGGCTTTTGTTGGTTTTAAAAGTCCATTGGAGACTATGGCGTCGTGCG 360
Elk328c3         GGGCCACCGTTTCGGCTTTTGTTGGTTTTAAAAGTCCATTGGAGATTATGGCGTCGTGCG 360
Elk416c8         GGGCCACCGTTTCGGCTTTTGTTGGTTTTAAAAGTCCATTGGAGATTATGGCGTCGTGCG 360
WTDN15c1         GGGCCACCGTTTCGGCTTTTGTTGGTTTTAAAAGTCCATTGGAGATTATGGCGTCGTGCG 360
WTDNL15c6        GGGCCACCGTTTCGGCTTTTGTTGGTTTTAAAAGTCCATTGGAGATTATGGCGTCGTGCG 360
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WTDN15c9         GGGCCACCGTTTCGGCTTTTGTTGGTTTTAAAAGTCCATTGGAGATTATGGCGTCGTGCG 360
WTDA21c4         GGGCCACCGTTTCGGCTTTTGTTGGTTTTAAAAGTCCATTGGAGATTATGGCGTCGTGCG 360
WTDA21c5         GGGCCACCGTTTCGGCTTTTGTTGGTTTTAAAAGTCCATTGGAGATTATGGCGTCGTGCG 360
WTDA21c6         GGGCCACCGTTTCGGCTTTTGTTGGTTTTAAAAGTCCATTGGAGATTATGGCGTCGTGCG 360
Cow139c10        GGGCCACCGTTTCGGCTTTTGTTGGTTTTAAAAGTCCATTGGAGATTATGGCGTCGTGCG 360
Cow139c11        GGGCCACCGTTTCGGCTTTTGTTGGTTTTAAAAGTCCATTGGAGATTATGGCGTCGTGCG 360
Cow2c4           GGGCCATCGTTTCGGCTTTTGTTGGTTTTAAAAGTCCATTGGAGATTATGGCGTCGTGCG 360
Cow133c1         GGGCCACCGTTTCGGCTTTTGTTGGTTTTAAAAGTCCATTGGAGATTATGGCGTCGTGCG 360
Cow139c9         GGGCCACCGTTTCGGCTTTTGTTGGTTTTAAAAGTCCATTGGAGATTATGGCGTCGTGCG 360
Cow3535c3        GGGCCACCGTTTCGGCTTTTGTTGGTTTTAAAAGTCCATTGGAGATTATGGCGTCGTGCG 360
Cow3535c6        GGGCCACCGTTTCGGCTTTTGTTGGTTTTAAAAGTCCATTGGAGATTATGGCGTCGTGCG 360
Cow2c7           GGGCCACCGTTTCGGCTTTTGTTGGTTTTAAAAGTCCATTGGAGATTATGGCGTCGTGCG 360
Cow2c10          GGGCCACCGTTTCGGCTTTTGTTGGTTTTAAAAGTCCATTGGAGATTATGGCGTCGTGCG 360
AB007814Tth      GGGCCACCGTTTCGGCTTTTGTTGGTTTTAAAAGTCCATTGGAGATTATGGCGTCGTGCG 360
Cow104c2         GGGCCACCGTTTCGGCTTTTGTTGGTTTTAAAAGTCCATTGGAGATTATGGCGTCGTGCG 360
Cow104c3         GGGCCACCGTTTCGGCTTTTGTTGGTTTTAAAAGTCCATTGGAGATTATGGCGTCGTGCG 360
Cow104c1         GGGCCACCGTTTCGGCTTTTGTTGGTTTTAAAAGTCCATTGGAGATTATGGCGTCGCGCG 360
Cow2095c4        GGGCCACCGTTTCGGCTTTTGTTGGTTTTAAAAGTCCATTGGAGATTATGGCGTCGTGCG 360
Cow2095c9        GGGCCACCGTTTCGGCTTTTGTTGGTTTTAAAAGTCCATTGGAGATTATGGCGTCGTGCG 360
Cow2095c8        GGGCCACCGTTTCGGCTTTTGTTGGTTTTAAAAGTCCATTGGAGATTATGGCGTCGTGCG 360
WTDA3c9.1        GGGCCACCGTTTCGGCTTTTGTTGGTTTTAAAAGTCCATTGGAGATTATGGCGTCGTGCG 360
WTDA3c9.4        GGGCCACCGTTTCGGCTTTTGTTGGTTTTAAAAGTCCATTGGAGATTATGGCGTCGTGCG 360
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Elk142c2         ACAAGCGTCTGGGTGTTTTCCCATTTTTGGGGCACCCGTCGCCTTTGCGGGAAATCCGTG 420
Elk142c10        ACAAGCGTCTGGGTCTTTTCCCATTTTTGGGGCACCCGTCGCCTTTGCGGGAAATCCGTG 420
Elk142c5         ACAAGCGTCTGGGTGTTTTCCCATTTTTGGGGCACCCGTCGCCTTTGCGGGAAATCCGTG 420
Elk328c3         ACAAGCGTCTGGGTGTTTTCCCATTTTTGGGGCACCCGTCGCCTTTGCGGGAAATCCGTG 420
Elk416c8         ACAAGCGTCTGGGTGTTTTCCCATTTTTGGGGCACCCGTCGCCTTTGCGGGAAATCCGTG 420
WTDN15c1         ACAAGCGTCTGGGTGTTTTCCCATTTT-GGGGCACCCGTCGCCTTTGCGGGAAATCCGTG 419
WTDNL15c6        ACAAGCGTCTGGGTGTTTTCCCATTTT-GGGGCACCCGTCGCCTTTGCGGGAAATCCGTG 419
WTDN15c9         ACAAGCGTCTGGGTGTTTTCCCATTTT-GGGGCACCCGTCGCCTTTGCGGGAAATCCGTG 419
WTDA21c4         ACAAGCGTCTGGGTGTTTTCCCATTTT-GGGGCACCCGTCGCCTTTGCGGGAAATCCGTG 419
WTDA21c5         ACAAGCGTCTGGGTGTTTTCCCATTTTTGGGGCACCCGTCGCCTTTGCGGGAAATCCGTG 420
WTDA21c6         ACAAGCGTCTGGGTGTTTTCCCATTTT-GGGGCACCCGTCGCCTTTGCGGGAAATCCGTG 419
Cow139c10        ACAAGCGTCTGGGTGTTTTCCCATCTTTGGGGCACCCGTCGCCTTTGCGAGAAATCCGTG 420
Cow139c11        ACAAGCGTCTGGGTGTTTTCCCATCTTTGGGGCACCCGTCGCCTTTGCGAGAAATCCGTG 420
Cow2c4           ACAAGCGTCTGGGTGTTTTCCCATCTTTGGGGCACCCGTCGCCTTTGCGAGAAATCCGTG 420
Cow133c1         ACAAGCGTCTGGGTGTTTTCCCATCTTTGGGGCACCCGTCGCCTTTGCGAGAAATCCGTG 420
Cow139c9         ACAAGCGTCTGGGTGTTTTCCCATCTTTGGGGCACCCGTCGCCTTTGCGAGAAACCCGTG 420
Cow3535c3        ACAAGCGTCTGGGTGTTTTCCCATCTTTGGGGCACCCGTCGCCTTTGCGAGAAATCCGTG 420
Cow3535c6        ACAAGCGTCTGGGTGTTTTCCCATCTTTGGGGCACCCGTCGCCTTTGCGAGAAATCCGTG 420
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Cow2c7          ACAAGCGTCTGGGTGTTTTCCCATCTTTGGGGCACCCGTCGCCTTTGTGAGAAATCCGTG 420
Cow2c10         ACAAGCGTCTGGGTGTTTTCCCATCTTTGGGGCACCCGTCGCCTTTGCGAGAAATCCGTG 420
AB007814Tth     ACAAGCGTCTGGGTGTTTTCCCATCTTTGGGGCACCCGTCGCCTTTGCGAGAAATCCGTG 420
Cow104c2        ACAAGCGTCTGGGTGTTTTCCCATCTTTGGGGCACCCGTCGCCTTTGCGAGAAATCCGTG 420
Cow104c3        ACAAGCGTCTGGGTGTTTTCCCATCTTTGGGGCACCCGTCGCCTTTGCGAGAAATCCGTG 420
Cow104c1        ACAAGCGTCTGGGTGTTTTCCCATCTTTGGGGCACCCGTCGCCTTTGCGAGAAATCCGTG 420
Cow2095c4       ACAAGCGTCTGGGTGTTTTCCCATCTTTGGGGCACCCGTCGCCTTTGCGAGAAATCCGTG 420
Cow2095c9       ACAAGCGTCTGGGTGTTTTCCCATCTTTGGGGCACCCGTCGCCTTTGCGAGAAATCCGTG 420
Cow2095c8       ACAAGCGTCTGGGTGTTTTCCCATCTTTGGGGCACCCGTCGCCTTTGCGAGAAATCCGTG 420
WTDA3c9.1       ACAAGCGTCTGGGTGATTCCCC--TCTTGGGGCGCCCGTCGCCTTTGCGGGAAATCCGTG 418
WTDA3c9.4       ACAAGCGTCTGGGTGATTCCCC--TCTTGGGGCGCCCGTCGCCTTTGCGGGAAATCCGTG 418
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Elk142c2        TCGGTGTTGATGAGCTTCGGCCCATCTCTCCGGCGCCTTCCCGTGACTCACGGCATCCAG 480
Elk142c10       TCGGTGTTGATGAGCTTCGGCCCATCTCTCCGGCGCCTTCCCGTGACTCACGGCATCCAG 480
Elk142c5        TCGGTGTTGATGAGCTTCGGCCCATCTCTCCGGCGCCTTCCCGTGACTCACGGCATCCAG 480
Elk328c3        TCGGTGTTGATGAGCTTCGGCCCATCTCTCCGGCGCCTTCCCGTGACTCACGGCATCCAG 480
Elk416c8        TCGGTGTTGATGAGCTTCGGCCCATCTCTCCGGCGCCTTCCCGTGACTCACGGCATCCAG 480
WTDN15c1        TCGGTGTTGATGAGCTTCGGCCCATCTCTCCGGCGCCTTCCCGTGACTCACGGCATCCAG 479
WTDNL15c6       TCGGTGTTGATGAGCTTCGGCCCATCTCTCCGGCGCCTTCCCGTGACTCACGGCATCCAG 479
WTDN15c9        TCGGTGTTGATGAGCTTCGGCCCATCTCTCCGGCGCCTTCCCGTGACTCACGGCATCCAG 479
WTDA21c4        TCGGTGTTGATGAGCTTCGGCCCATCTCTCCGGCGCCTTCCCGTGACTCACGGCATCCAG 479
WTDA21c5        TCGGTGTTGATGAGCTTCGGCCCATCTCTCCGGCGCCTTCCCGTGACTCACGGCATCCAA 480
WTDA21c6        TCGGCGTTGATGAGCTTCGGCCCATCTCTCCGGCGCCTTCCCGTGACTCACGGCATCCAG 479
Cow139c10       TCGGTGTTGATGAGCTTCGGCCCATCTCTCCGGCGCCTTCTCGTGACTCACGGCATCCAG 480
Cow139c11       TCGGTGTTGATGAGCTTCGGCCCATCTCTCCGGCGCCTTCTCGTGACTCACGGCATCCAG 480
Cow2c4          TCGGTGTTGATGAGCTTCGGCCCATCTCTCCGGCGCCTTCTCGTGACTCACGGCATCCAG 480
Cow133c1        TCGGTGTTGATGAGCTTCGGCCCATCTCTCCGGCGCCTTCTCGTGACTCACGGCATCCAG 480
Cow139c9        TCGGTGTTGATGAGCTTCGGCCCATCTCTCCGGCGCCTTCTCGTGACTCACGGCATCCAG 480
Cow3535c3       TCGGTGTTGATGAGCTTCGGCCCATCTCTCCGGCGCCTTCTCGTGACTCACGGCATCCAG 480
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Cow2c7          TCGGTGTTGATGAGCTTCGGCCCATCTCTCCGGCGCCTTCTCGTGACTCACGGCATCCAG 480
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AB007814Tth     TCGGTGTTGATGAGCTTCGGCCCATCTCTCCGGCGCCTTCTCGTGACTCACGGCATCCAG 480
Cow104c2        TCGGTGTTGATGAGCTTCGGCCCATCTCTCCGGCGCCTTCTCGTGACTCACGGCATCCAG 480
Cow104c3        TCGGTGTTGATGAGCTTCGGCCCATCTCTCCGGCGCCTTCTCGTGACTCACGGCATCCAG 480
Cow104c1        TCGGTGTTGATGAGCTTCGGCCCATCTCTCCGGCGCCTTCTCGTGACTCACGGCATCCAG 480
Cow2095c4       TCGGTGTTGATGAGCTTCGGCCCATCTCTCCGGCGCCTTCTCGTGACTCACGGCATCCAG 480
Cow2095c9       TCGGTGTTGATGAGCTTCGGCCCATCTCTCCGGCGCCTTCTCGTGACTCACGGCATCCAG 480
Cow2095c8       TCGGTGTTGATGAGCTTCGGCCCATCTCTCCGGCGCCTTCTCGTGACTCACGGCATCCAG 480
WTDA3c9.1       TCGGTGTTGATGAGCTTCGGCCCATCTCTCCGGCGCCTTCCCGTGACTCACGGCATCCAG 478
WTDA3c9.4       TCGGTGTTGATGAGCTTCGGCCCATCTCTCCGGCGCCTTCCCGTGACTCACGGCATCCAG 478
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Elk142c2          GAATGAAGGAGGGTAGTTCGGGGG--AGAACGTACTGGCGCGTCAGAGGTGAAATTCTTA 538
Elk142c10         GAATGAAGGAGGGTAGTTCGGGGG--AGAACGTACTGGCGCGTCAGAGGTGAAATTCTTA 538
Elk142c5          GAATGAAGGAGGGTAGTTCGGGGG--AGAACGTACTGGCGCGTCAGAGGTGAAATTCTTA 538
Elk328c3          GAATGAAGGAGGGTAGTTCGGGGG--AGAACGTACTGGCGCGTCAGAGGTGAAATTCTTA 538
Elk416c8          GAATGAAGGAGGGTAGTTCGGGGG--AGAACGTACTGGCGCGTCAGAGGTGAAATTCTTA 538
WTDN15c1          GAATGAAGGAGGGTAGTTCGGGGG--AGAACGTACTGGCGCGTCAGAGGTGAAATTCTTA 537
WTDNL15c6         GAATGAAGGAGGGTAGTTCGGGGG--AGAACGTACTGGCGCGTCAGAGGTGAAATTCTTA 537
WTDN15c9          GAATGAAGGAGGGTAGTTCGGGGG--AGAACGTACTGGCGCGTCAGAGGTGAAATTCTTA 537
WTDA21c4          GAATGAAGGAGGGTAGTTCGGGGGGGAGAACGTACTGGCGCGTCAGAGGTGAAATTCTTA 539
WTDA21c5          GAATGAAGGAGGGTAGTTCGGGGG--AGAACGTACTGGCGCGTCAGAGGTGAAATTCTTA 538
WTDA21c6          GAATGAAGGAGGGTAGTTCGGGGG--AGAACGTACTGGCGCGTCAGAGGTGAAATTCTTA 537
Cow139c10         GAATGAAGGAGGGTAGTTCGGGGG--AGAACGTACTGGCGCGTCAGAGGTGAAATTCTTA 538
Cow139c11         GAATGAAGGAGGGTAGTTCGGGGG--AGAACGTACTGGCGCGTCAGAGGTGAAATTCTTA 538
Cow2c4            GAATGAAGGAGGGTAGTTCGGGGG--AGAACGTACTGGCGCGTCAGAGGTGAAATTCTTA 538
Cow133c1          GAATGAAGGAGGGTAGTTCGGGGG--AGAACGTACTGGCGCGTCAGAGGTGAAATTCTTA 538
Cow139c9          GAATGAAGGAGGGTAGTTCGGGGG--AGAACGTACTGGCGCGTCAGAGGTGAAATTCTTA 538
Cow3535c3         GAATGAAGGAGGGTAGTTCGGGGG--AGAACGTACTGACGCGTCAGAGGTGAAATTCTTA 538
Cow3535c6         GAATGAAGGAGGGTAGTTCGGGGG--AGAACGTACTGGCGCGTCAGAGGTGAAATTCTTA 538
Cow2c7            GAATGAAGGAGGGTAGTTCGGGGG--AGAACGTACTGGCGCGTCAGAGGTGAAATTCTTA 538
Cow2c10           GAATGAAGGAGGGTAGTTCGGGGG--AGAACGTACTGGCGCGTCAGAGGTGAAATTCTTA 538
AB007814Tth       GAATGAAGGAGGGTAGTTCGGGGG--AGAACGTACTGGCGCGTCAGAGGTGAAATTCTTA 538
Cow104c2          GAATGAAGGAGGGTAGTTCGGGGG--AGAACGTACTGGCGCGTCAGAGGTGAAATTCTTA 538
Cow104c3          GAATGAAGGAGGGTAGTTCGGGGG--AGAACGTACTGGCGCGTCAGAGGTGAAATTCTTA 538
Cow104c1          GAATGAAGGAGGGTAGTTCGGGGG--AGAACGTACTGGCGCGTCAGAGGTGAAATTCTTA 538
Cow2095c4         GAATGAAGGAGGGTAGTTCGGGGG--AGAACGTACTGGCGCGTCAGAGGTGAAATTCTTA 538
Cow2095c9         GAATGAAGGAGGGTAGTTCGGGGG--AGAACGTACTGGCGCGTCAGAGGTGAAATTCTTA 538
Cow2095c8         GAATGAAGGAGGGTAGTTCGGGGG--AGAACGTACTGGCGCGTCAGAGGTGAAATTCTTA 538
WTDA3c9.1         GAATGAAGGAGGGTAGTTCGGGGG--AGAACGTACTGGCGCGTCAGAGGTGAAATTCTTA 536
WTDA3c9.4         GAATGAAGGAGGGTAGTTCGGGGG--AGAACGTACTGGCGCGTCAGAGGTGAAATTCTTA 536
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Elk142c2          GACCGCGCCAAGACGAACTACAGCGAAGGCATTCTTCAAGGATACCTTCCTCAATCAAGA 598
Elk142c10         GACCGCGCCAAGACGAACTACAGCGAAGGCATTCTTCAAGGATACCTTCCTCAATCAAGA 598
Elk142c5          GACCGCGCCAAGACGAACTACAGCGAAGGCATTCTTCAAGGATACCTTCCTCAATCAAGA 598
Elk328c3          GACCGCGCCAAGACGAACTACAGCGAAGGCATTCTTCAAGGATACCTTCCTCAATCAAGA 598
Elk416c8          GACCGCGCCAAGACGAACTACAGCGAAGGCATTCTTCAAGGATACCTTCCTCAATCAAGA 598
WTDN15c1          GACCGCGCCAAGACGAACTACAGCGAAGGCATTCTTCAAGGATACCTTCCTCAATCAAGA 597
WTDNL15c6         GACCGCGCCAAGACGAACTACAGCGAAGGCATTCTTCAAGGATACCTTCCTCAATCAAGA 597
WTDN15c9          GACCGCGCCAAGACGAACTACAGCGAAGGCATTCTTCAAGGATACCTTCCTCAATCAAGA 597
WTDA21c4          GACCGCGCCAAGACGAACTACAGCGAAGGCATTCTTCAAGGATACCTTCCTCAATCAAGA 599
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WTDA21c5       GACCGCGCCAAGACGAACTACAGCGAAGGCATTCTTCAAGGATACCTTCCTCAATCAAGA 598
WTDA21c6       GACCGCGCCAAGACGAACTACAGCGAAGGCATTCTTCAAGGATACCTTCCTCAATCAAGA 597
Cow139c10      GACCGCGCCAAGACGAACTACAGCGAAGGCATTCTTCAAGGATACCTTCCTCAATCAAGA 598
Cow139c11      GACCGCGCCAAGACGAACTACAGCGAAGGCATTCTTCAAGGATACCTTCCTCAATCAAGA 598
Cow2c4         GACCGCGCCAAGACGAACTACAGCGAAGGCATTCTTCAAGGATACCTTCCTCAATCAAGA 598
Cow133c1       GACCGCGCCAAGACGAACTACAGCGAAGGCATTCTTCAAGGATACCTTCCCCAATCAAGA 598
Cow139c9       GACCGCGCCAAGACGAACTACAGCGAAGGCATTCTTCAAGGATACCTTCCTCAATCAAGA 598
Cow3535c3      GACCGCGCCAAGACGAACTACAGCGAAGGCATTCTTCAAGGATACCTTCCTCAATCAAGA 598
Cow3535c6      GACCGCGCCAAGACGAACTACAGCGAAGGCATTCTTCAAGGATACCTTCCTCAATCAAGA 598
Cow2c7         GACCGCGCCAAGACGAACTACAGCGAAGGCATTCTTCAAGGATACCTTCCTCAATCAAGA 598
Cow2c10        GACCGCGCCAAGACGAACTACAGCGAAGGCATTCTTCAAGGATACCTTCCTCAATCAAGA 598
AB007814Tth    GACCGCGCCAAGACGAACTACAGCGAAGGCATTCTTCAAGGATACCTTCCTCAATCAAGA 598
Cow104c2       GACCGCGCCAAGACGAACTACAGCGAAGGCATTCTTCAAGGATACCTTCCTCAATCAAGA 598
Cow104c3       GACCGCGCCAAGACGAACTACAGCGAAGGCATTCTTCAAGGATACCTTCCTCAATCAAGA 598
Cow104c1       GACCGCGCCAAGACGAACTACAGCGAAGGCATTCTTCAAGGATACCTTCCTCAATCAAGA 598
Cow2095c4      GACCGCGCCAAGACGAACTACAGCGAAGGCATTCTTCAAGGATACCTTCCTCAATCAAGA 598
Cow2095c9      GACCGCGCCAAGACGAACTACAGCGAAGGCATTCTTCAAGGATACCTTCCTCAATCAAGA 598
Cow2095c8      GACCGCGCCAAGACGAACTACAGCGAAGGCATTCTTCAAGGATACCTTCCTCAATCAAGA 598
WTDA3c9.1      GACCGCGCCAAGACGAACTACAGCGAAGGCATTCTTCAAGGATACCTTCCTCAATCAAGA 596
WTDA3c9.4      GACCGCGCCAAGACGAACTACAGCGAAGGCATTCTTCAAGGATACCTTCCTCAATCAAGA 596
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Elk142c2       ACCAAAGTGTGGGGATCGAAGATGATTAGAGACCATTGTAGTCCACACTGCAAACGATGA 658
Elk142c10      ACCAAAGTGTGGGGATCGAAGATGATTAGAGACCATTGTAGTCCACACTGCAAACGATGA 658
Elk142c5       ACCAAAGTGTGGGGATCGAAGATGATTAGAGACCATTGTAGTCCACACTGCAAACGATGA 658
Elk328c3       ACCAAAGTGTGGGGATCGAAGATGATTAGAGACCATTGTAGTCCACACTGCAAACGATGA 658
Elk416c8       ACCAAAGTGTGGGGATCGAAGATGATTAGAGGCCATTGTAGTCCACACTGCAAACGATGA 658
WTDN15c1       ACCAAAGTGTGGGGATCGAAGATGATTAGAGACCATTGTAGTCCACACTGCAAACGATGA 657
WTDNL15c6      ACCAAAGTGTGGGGATCGAAGATGATTAGAGACCATTGTAGTCCACACTGCAAACGATGA 657
WTDN15c9       ACCAAAGTGTGGGGATCGAAGATGATTAGAGACCATTGTAGTCCACACTGCAAACGATGA 657
WTDA21c4       ACCAAAGTGTGGGGATCGAAGATGATTAGAGACCATTGTAGTCCACACTGCAAACGATGA 659
WTDA21c5       ACCAAAGTGTGGGGATCGAAGATGATTAGAGACCATTGTAGTCCACACTGCAAACGATGA 658
WTDA21c6       ACCAAAGTGTGGGGATCGAAGATGATTAGAGACCATTGTAGTCCACACTGCAAACGATGA 657
Cow139c10      ACCAAAGTGTGGGGATCGAAGATGATTAGAGACCATTGTAGTCCACACTGCAAACGATGA 658
Cow139c11      ACCAAAGTGTGGGGATCGAAGATGATTAGAGACCATTGTAGTCCACACTGCAAACGATGA 658
Cow2c4         ACCAAAGTGTGGGGATCGAAGATGATTAGAGACCATTGTAGTCCACACTGCAAACGATGA 658
Cow133c1       ACCAAAGTGTGGGGATCGAAGATGATTAGAGACCATTGTAGTCCACACTGCAAACGATGA 658
Cow139c9       ACCAAAGTGTGGGGATCGAAGATGATTAGAGACCATTGTAGTCCACACTGCAAACGATGA 658
Cow3535c3      ACCAAAGTGTGGGGATCGAAGATGATTAGAGACCATTGTAGTCCACACTGCAAACGATGA 658
Cow3535c6      ACCAAAGTGTGGGGATCGAAGATGATTAGGGACCATTGTAGTCCACACTGCAAACGATGA 658
Cow2c7         ACCAAAGTGTGGGGATCGAGGATGATTAGAGACCATTGTAGTCCACACTGCAAACGATGA 658
Cow2c10        ACCAAAGTGTGGGGATCGAAGATGATTAGAGACCATTGTAGTCCACACTGCAAACGATGA 658
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AB007814Tth      ACCAAAGTGTGGGGATCGAAGATGATTAGAGACCATTGTAGTCCACACTGCAAACGATGA 658
Cow104c2         ACCAAAGTGTGGGGATCGAAGATGATTAGAGACCATTGTAGTCCACACTGCAAACGATGA 658
Cow104c3         ACCAAAGTGTGGGGATCGAAGATGATTAGAGACCATTGTAGTCCACACTGCAAACGATGA 658
Cow104c1         ACCAAAGTGTGGGGATCGAAGATGATTAGAGACCATTGTAGTCCACACTGCAAACGATGA 658
Cow2095c4        ACCAAAGTGTGGGGATCGAAGATGATTAGAGACCATTGTAGTCCACACTGCAAACGATGA 658
Cow2095c9        ACCAAAGTGTGGGGGTCGAAGATGATTAGAGACCATTGTAGTCCACACTGCAAACGATGA 658
Cow2095c8        ACCAAAGTGTGGGGATCGAAGATGATTAGAGACCATTGTAGTCCACACTGCAAACGATGA 658
WTDA3c9.1        ACCAAAGTGTGGGGATCGAAGATGATTAGAGACCATTGTAGTCCACACTGCAAACGATGA 656
WTDA3c9.4        ACCAAAGTGTGGGGATCGAAGATGATTAGAGACCATTGTAGTCCACACTGCAAACGATGA 656
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Elk142c2         CACCCATGAATTGGGGAATTTTTGGTCGTAGGCGAGGTCGGGTTCATCTCGCTCCTCGCC 718
Elk142c10        CACCCATGAATTGGGGAATTTTTGGTCGTAGGCGAGGTCGGGTTCATCTCGCTCCTCGCC 718
Elk142c5         CACCCATGAATTGGGGAATTTT-GGTCGTAGGCGAGGTCGGGTTCATCTCGCTCCTCGCC 717
Elk328c3         CACCCATGAATTGGGGAATTTTTGGTCGTAGGCGAGGTCGGGTTCATCTCGCTCCTCGCC 718
Elk416c8         CACCCATGAATTGGGGAATTTTTGGTCGTAGGCGAGGTCGGGTTCATCTCGCTCCTCGCC 718
WTDN15c1         CACCCATGAATTGGGGAATTTTTGGTCGTAGGCGAGGTCGGGTTCATCTCGCTCCTCGCC 717
WTDNL15c6        CACCCATGAATTGGGGAATTTTTGGTCGTAGGCGAGGTCGGGTTCATCTCGCTCCTCGCC 717
WTDN15c9         CACCCATGAATTGGGGAATTTTTGGTCGTAGGCGAGGTCGGGTTCATCTCGCTCCTCGCC 717
WTDA21c4         CACCCATGAATTGGGGAATTTTTGGTCGTAGGCGAGGTCGGGTTCATCTCGCTCCTCGCC 719
WTDA21c5         CACCCATGAATTGGGGAATTTTTGGTCGTAGGCGAGGTCGGGTTCATCTCGCTCCTCGCC 718
WTDA21c6         CACCCATGAATTGGGGAATTTTTGGTCGTAGGCGAGGTCGGGTTCATCTCGCTCCTCGCC 717
Cow139c10        CACCCATGAATTGGGGAATTTTTGGTCGTAGGCGAGGTCGGGTTCATCTCGCTCCTCGCC 718
Cow139c11        CACCCATGAATTGGGGAATTTTTGGTCGTAGGCGAGGTCGGGTTCATCTCGCTCCTCGCC 718
Cow2c4           CACCCATGAATTGGGGAATTTTTGGTCGTAGGCGAGGTCGGGTTCATCTCGCTCCTCGCC 718
Cow133c1         CACCCATGAATTGGGGAATTTTTGGTCGTAGGCGAGGTCGGGCTCATCTCGCTCCTCGCC 718
Cow139c9         CACCCATGAATTGGGGAATTTTTGGTCGTAGGCGAGGTCGGGTTCATCTCGCTCCTCGCC 718
Cow3535c3        CACCCATGAATTGGGGAATTTTTGGTCGTAGGCGAGGTCGGGTTCATCTCGCTCCTCGCC 718
Cow3535c6        CACCCATGAATTGGGGAATTTTTGGTCGTAGGCGAGGTCGGGTTCACCTCGCTCCTCGCC 718
Cow2c7           CACCCATGAATTGGGGAATTTTTGGTCGTAGGCGAGGTCGGGTTCATCTCGCTCCTCGCC 718
Cow2c10          CACCCATGAATTGGGGAATTTTTGGTCGTAGGCGAGGTCGGGTTCATCTCGCTCCTCGCC 718
AB007814Tth      CACCCATGAATTGGGGAATTTTTGGTCGTAGGCGAGGTCGGGTTCATCTCGCTCCTCGCC 718
Cow104c2         CACCCATGAATTGGGGAATTTTTGGTCGTAGGCGAGGTCGGGTTCATCTCGCTCCTCGCC 718
Cow104c3         CACCCATGAATTGGGGAATTTTTGGTCGTAGGCGAGGTCGGGTTCATCTCGCTCCTCGCC 718
Cow104c1         CACCCATGAATTGGGGAATTTTTGGTTGTAGGCGAGGTCGGGTTCATCTCGCTCCTCGCC 718
Cow2095c4        CACCCATGAATTGGGGAATTTTTGGTCGTAGGCGAGGTCGGGTTCATCTCGCTCCTCGCC 718
Cow2095c9        CACCCATGAATTGGGGAATTTTTGGTCGTAGGCGAGGTCGGGTTCATCTCGCTCCTCGCC 718
Cow2095c8        CACCCATGAATTGGGGAATTTTTGGTCGTAGGCGAGGTCGGGTTCATCTCGCTCCTCGCC 718
WTDA3c9.1        CACCCATGAATTGGGGAATTTTTGGTCGTAGGCGGGGTCGAGTTCATCTCGCTCCTCGCC 716
WTDA3c9.4        CACCCATGAATTGGGGAATTTTTGGTCGTAGGCGGGGTCGAGTTCATCTCGCTCCTCGCC 716
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Elk142c2        TCGCCAATGGATATCAATTTACGTGCATATTCTTTTCGGTCCTCGCAAGGGGGCCTTTAA 778
Elk142c10       TCGCCAATGGATATCAATTTACGTGCATATTCTTTTCGGTCCTCGCAAGGGGGCCTTTAA 778
Elk142c5        TCGCCAATGGATATCAATTTACGTGCATATTCTTTTCGGTCCTCGCAAGGGGGCCTTTAA 777
Elk328c3        TCGCCAATGGATATCAATTTACGTGCATATTCTTTTCGGTCCTCGCAAGGGGGCCTTTAA 778
Elk416c8        TCGCCAATGGATATCAATTTACGTGCATATTCTTTTCGGTCCTCGCAAGGGGGCCTTTAA 778
WTDN15c1        TCGCCAATGGATATCAATTTACGTGCATATTCTTTTCGGTCCTCGCAAGGGGGCCTTTAA 777
WTDNL15c6       TCGCCAATGGATATCAATTTACGTGCATATTCTTTTCGGTCCTCGCAAGGGGGCCTTTAA 777
WTDN15c9        TCGCCAATGGATATCAATTTACGTGCATATTCTTTTCGGTCCTCGCAAGGGGGCCTTTAA 777
WTDA21c4        TCGCCAATGGATATCAATTTACGTGCATATTCTTTTCGGTCCTCGCAAGGGGGCCTTTAA 779
WTDA21c5        TCGCCAATGGATATCAATTTACGTGCATATTCTTTTCGGTCCTCGCAAGGGGGCCTTTAA 778
WTDA21c6        TCGCCAATGGATATCAATTTACGTGTATATTCTTTTCGGTCCTCGCAAGGGGGCCTTTAA 777
Cow139c10       TCGCCAATGGATATCAATTTACGTGCATATTCTTTTCGGTCCTCGCAAGGGGGCCTTTAA 778
Cow139c11       TCGCCAATGGATATCAATTTACGTGCATATTCTTTTCGGTCCTCGCAAGGGGGCCTTTAA 778
Cow2c4          TCGCCAATGGATATCAATTTACGTGCATATTCTTTTCGGTCCTCGCAAGGGGGCCTTTAA 778
Cow133c1        TCGCCAATGGATATCAATTTACGTGCATATTCTTTTCGGTCCTCGCAAGGGGGCCTTTAA 778
Cow139c9        TCGCCAATGGATATCAATTTACGTGCATATTCTTTTCGGTCCTCGCAAGGGGGCCTTTAA 778
Cow3535c3       TCGCCAATGGATATCAATTTACGTGCATATTCTTTTCGGTCCTCGCAAGGGGGCCTTTAA 778
Cow3535c6       TCGCCAATGGATATCAATTTACGTGCATATTCTTTTCGGTCCTCGCAAGGGGGCCTTTAA 778
Cow2c7          TCGCCAATGGATATCAATTTACGTGCATATTCTTTTCGGTCCTCGCAAGGGGGCCTTTAA 778
Cow2c10         TCGCCAATGGATATCAATTTACGTGCATATTCTTTTCGGTCCTCGCAAGGGGGCCTTTAA 778
AB007814Tth     TCGCCAATGGATATCAATTTACGTGCATATTCTTTTCGGTCCTCGCAAGGGGGCCTTTAA 778
Cow104c2        TCGCCAATGGATATCAATTTACGTGCATATTCTTTTCGGTCCTCGCAAGGGGGCCTTTAA 778
Cow104c3        TCGCCAATGGATATCAATTTACGTGCATATTCTTTTCGGTCCTCGCAAGGGGGCCTTTAA 778
Cow104c1        TCGCCAATGGATATCAATTTACGTGCATATTCTTTTCGGTCCTCGCAAGGGGGCCTTTAA 778
Cow2095c4       TCGCCAATGGATATCAATTTACGTGCATATTCTTTTCGGTCCTCGCAAGGGGGCCTTTAA 778
Cow2095c9       TCGCCAATGGATATCAATTTACGTGCATATTCTTTTCGGTCCTCGCAAGGGGGCCTTTAA 778
Cow2095c8       TCGCCAATGGATATCAATTTACGTGCATATTCTTTTCGGTCCTCGCAAGGGGGCCTTTAA 778
WTDA3c9.1       TCGCCAATGGATATCAATTTACGTGCATATTCTTTTCGGTCCTCGCAAGGGGGCCTTTAA 776
WTDA3c9.4       TCGCCAATGGATATCAATTTACGTGCATATTCTTTTCGGTCCTCGCAAGGGGGCCTTTAA 776
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Elk142c2        CGGGAATATCCTCAGCACGTTATCTGACTTCTTCACGCGAAAGCTTTGAGGTTACAGTCT 838
Elk142c10       CGGGAATATCCTCAGCACGTTATCTGACTTCTTCACGCGAAAGCTTTGAGGTTACAGTCT 838
Elk142c5        CGGGAATATCCTCAGCACGTTATCTGACTTCTTCACGCGAAAGCTTTGAGGTTACAGTCT 837
Elk328c3        CGGGAATATCCTCAGCACGTTATCTGACTTCTTCACGCGAAAGCTTTGAGGTTACAGTCT 838
Elk416c8        CGGGAATATCCTCAGCACGTTATCTGACTTCTTCACGCGAAAGCTTTGAGGTTACAGTCT 838
WTDN15c1        CGGGAATATCCTCAGCACGTTATCTGACTTCTTCACGCGAAAGCTTTGAGGTTACAGTCT 837
WTDNL15c6       CGGGAATATCCTCAGCACGTTATCTGACTTCTTCACGCGAAAGCTTTGAGGTTACAGTCT 837
WTDN15c9        CGGGAATATCCTCAGCACGTTATCTGACTTCTTCACGCGAAAGCTTTGAGGTTACAGCCT 837
WTDA21c4        CGGGAATATCCTCAGCACGTTATCTGACTTCTTCACGCGAAAGCTTTGAGGTTACAGTCT 839
WTDA21c5        CGGGAATATCCTCAGCACGTTATCTGACTTCTTCACGCGAAAGCTTTGAGGTTACAGTCT 838
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WTDA21c6        CGGGAATATCCTCAGCACGTTATCTGACTTCTTCACGCGAAAGCTTTGAGGTTACAGTCT 837
Cow139c10       CGGGAATATCCTCAGCACGTTATCTGACTTCTTCACGCGAAAGCTTTGAGGTTACAGTCT 838
Cow139c11       CGGGAATATCCTCAGCACGTTATCTGACTTCTTCACGCGAAAGCTTTGAGGTTACAGTCT 838
Cow2c4          CGGGAATATCCTCAGCACGTTATCTGACTTCTTCATGCGAAAGCTTTGAGGTTACAGTCT 838
Cow133c1        CGGGAATATCCTCAGCACGTTATCTGACTTCTTCACGCGAAAGCTTTGAGGTTACAGTCT 838
Cow139c9        CGGGAATATCCTCAGCACGTTATCTGACTTCTTCACGCGAAAGCTTTGAGGTTACAGTCT 838
Cow3535c3       CGGGAATATCCTCAGCACGTTATCTGACTTCTTCACGCGAAAGCTTTGAGGTTACAGTCT 838
Cow3535c6       CGGGAATATCCTCAGCACGTTATCTGACTTCTTCACGCGAAAGCTTTGAGGTTACAGTCT 838
Cow2c7          CGGGAATATCCTCAGCACGTTATCTGACTTCTTCACGCGAAAGCTTTGAGGTTACAGTCT 838
Cow2c10         CGGGAATATCCTCAGCACGTTATCTGACTTCTTCACGCGAAAGCTTTGAGGTTACAGTCT 838
AB007814Tth     CGGGAATATCCTCAGCACGTTATCTGACTTCTTCACGCGAAAGCTTTGAGGTTACAGTCT 838
Cow104c2        CGGGAATATCCTCAGCACGTTATCTGACTTCTTCACGCGAAAGCTTTGAGGTTACAGTCT 838
Cow104c3        CGGGAATATCCTCAGCACGTTATCTGACTTCTTCACGCGAAAGCTTTGAGGTTACAGTCT 838
Cow104c1        CGGGAATATCCTCAGCACGTTATCTGACTTCTTCACGCGAAAGCTTTGAGGTTACAGTCT 838
Cow2095c4       CGGGAATATCCTCAGCACGTTATCTGACTTCTTCACGCGAAAGCTTTGAGGTTACAGTCT 838
Cow2095c9       CGGGAATATCCTCAGCACGTTATCTGACTTCTTCACGCGAAAGCTTTGAGGTTACAGTCT 838
Cow2095c8       CGGGAATATCCTCAGCACGTTATCTGACTTCTTCACGCGAAAGCTTTGAGGTTACAGTCT 838
WTDA3c9.1       CGGGAATATCCTCAGCACGTTATCTGACTCCTTCACGCGAAAGCTTTGAGGTTACAGTCT 836
WTDA3c9.4       CGGGAATATCCTCAGCACGTTATCTGACTCCTTCACGCGAAAGCTTTGAGGTTACAGTCT 836
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Elk142c2        CAGGGGGGAGTACGTTCGCAAGAGTGAAACTTAAAGAAATTGACGGAATGGCACCACAAG 898
Elk142c10       CAGGGGGGAGTACGTTCGCAAGAGTGAAACTTAAAGAAATTGACGGAATGGCACCACAAG 898
Elk142c5        CAGGGGGGAGTACGTTCGCAAGAGTGAAACTTAAAGAAATTGACGGAATGGCACCACAAG 897
Elk328c3        CAGGGGGGAGTACGTTCGCAAGAGTGAAACTTAAAGAAATTGACGGAATGGCACCACAAG 898
Elk416c8        CAGGGGGGAGTACGTTCGCAAGAGTGAAACTTAAAGAAATTGACGGAATGGCACCACAAG 898
WTDN15c1        CAGGGGGGAGTACGTTCGCAAGAGTGAAACTTAAAGAAATTGACGGAATGGCACCACAAG 897
WTDNL15c6       CAGGGGGGAGTACGTTCGCAAGAGTGAAACTTAAAGAAATTGACGGAATGGCACCACAAG 897
WTDN15c9        CAGGGGGGAGTACGTTCGCAAGAGTGAAACTTAAAGAAATTGACGGAATGGCACCACAAG 897
WTDA21c4        C-GGGGGGAGTACGTTCGCAAGAGTGAAACTTAAAGAAATTGACGGAATGGCACCACAAG 898
WTDA21c5        C-GGGGGGAGTACGTTCGCAAGAGTGAAACTTAAAGAAATTGACGGAATGGCACCACAAG 897
WTDA21c6        C-GGGGGGAGTACGTTCGCAAGAGTGAAACTTAAAGAAATTGACGGAATGGCACCACAAG 896
Cow139c10       CAGGGGGGGGTACGTTCGCAAGAGTGAAACTTAAAGAAATTGACGGAATGGCACCACAAG 898
Cow139c11       CAGGGGGGGGTACGTTCGCAAGAGTGAAACTTAAAGAAATTGACGGAATGGCACCACAAG 898
Cow2c4          CAGGGGGGAGTACGTTCGCAAGAGTGAAACTTAAAGAAATTGACGGAATGGCACCACAAG 898
Cow133c1        CAGGGGGGAGTACGTTCGCAAGAGTGAAACTTAAAGAAATTGACGGAATGGCACCACAAG 898
Cow139c9        CAGGGGGGAGTACGTTCGCAAGAGTGAAACTTAAAGAAATTGACGGAATGGCACCACAAG 898
Cow3535c3       CAGGGGGGAGTACGTTCGCAAGAGTGAAACTTAAAGAAATTGACGGAATGGCACCACAAG 898
Cow3535c6       CAGGGGGGAGTACGTTCGCAAGAGTGAAACTTAAAGAAATTGACGGAATGGCACCACAAG 898
Cow2c7          CAGGGGGGAGTACGTTCGCAAGAGTGAAACTTAAAGAAATTGACGGAATGGCACCACAAG 898
Cow2c10         CAGGGGGGAGTACGTTCGCAAGAGTGAAACTTAAAGAAATTGACGGAATGGCACCACAAG 898
AB007814Tth     CAGGGGGGAGTACGTTCGCAAGAGTGAAACTTAAAGAAATTGACGGAATGGCACCACAAG 898
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Cow104c3         CAGGGGGGAGTACGTTCGCAAGAGTGAAACTTAAAGAAATTGACGGAATGGCACCACAAG 898
Cow104c1         CAGGGGGGAGTACGTTCGCAAGAGTGAAACTTAAAGAAATTGACGGAATGGCACCACGAG 898
Cow2095c4        CAGGGGGGAGTACGTTCGCAAGAGTGAAACTTAAAGAAATTGACGGAATGGCACCACAAG 898
Cow2095c9        CAGGGGGGAGTACGTTCGCAAGAGTGAAACTTAAAGAAATTGACGGAATGGCACCACAAG 898
Cow2095c8        CAGGGGGGAGTACGTTCGCAAGAGTGAAACTTAAAGAAATTGACGGAATGGCACCACAAG 898
WTDA3c9.1        CAGGGGGGAGTACGTTCGCAAGAGTGAAACTTAAAGAAATTGACGGAATGGCACCACAAG 896
WTDA3c9.4        CAGGGGGGAGTACGTTCGCAAGAGTGAAACTTAAAGAAATTGACGGAATGGCACCACAAG 896
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Elk142c2         ACGTGGAGCGTGCGGTTTAATTTGACTCAACACGGGGAACTTTACCAGATCCGGACAGGG 958
Elk142c10        ACGTGGAGCGTGCGGTTTAATTTGACTCAACACGGGGAACTTTACCAGATCCGGACAGGG 958
Elk142c5         ACGTGGAGCGTGCGGTTTAATTTGACTCAACACGGGGAACTTTACCAGATCCGGACAGGG 957
Elk328c3         ACGTGGAGCGTGCGGTTTAATTTGACTCAACACGGGGAACTTTACCAGATCCGGACAGGG 958
Elk416c8         ACGTGGAGCGTGCGGTTTAATTTGACTCAACACGGGGAACTTTACCAGATCCGGACAGGG 958
WTDN15c1         ACGTGGAGCGTGCGGTTTAATTTGACTCAACACGGGGAACTTTACCAGATCCGGACAGGG 957
WTDNL15c6        ACGTGGAGCGTGCGGTTTAATTTGACTCAACACGGGGAACTTTACCAGATCCGGACAGGG 957
WTDN15c9         ACGTGGAGCGTGCGGTTTAATTTGACTCAACACGGGGAACTTTACCAGATCCGGACAGGG 957
WTDA21c4         ACGTGGAGCGTGCGGTTTAATTTGACTCAACACGGGGAACTTTACCAGATCCGGACAGGG 958
WTDA21c5         ACGTGGAGCGTGCGGTTTAATTTGACTCAACACGGGGAACTTTACCAGATCCGGACAGGG 957
WTDA21c6         ACGTGGAGCGTGCGGTTTAATTTGACTCAACACGGGGAACTTTACCAGATCCGGACAGGG 956
Cow139c10        ACGTGGAGCGTGCGGTTTAATTTGACTCAACACGGGGAACTTTACCAGATCCGGACAGGG 958
Cow139c11        ACGTGGAGCGTGCGGTTTAATTTGACTCAACACGGGGAACTTTACCAGATCCGGACAGGG 958
Cow2c4           ACGTGGAGCGTGCGGTTTAATTTGACTCAACACGGGGAACTTTACCAGATCCGGACAGGG 958
Cow133c1         ACGTGGAGCGTGCGGTTTAATTTGACTCAACACGGGGAACTTTACCAGATCCGGACAGGG 958
Cow139c9         ACGTGGAGCGTGCGGTTTAATTTGACTCAACACGGGGAACTTTACCAGATCCGGACAGGG 958
Cow3535c3        ACGCGGAGCGTGCGGTTTAATTTGACTCAACACGGGGAACTTTACCAGATCCGGACAGGG 958
Cow3535c6        ACGTGGAGCGTGCGGTTTAATTTGACTCAACACGGGGAACTTTACCAGATCCGGACAGGG 958
Cow2c7           ACGTGGAGCGTGCGGTTTAATTTGACTCAACACGGGGAACTTTGCCAGATCCGGACAGGG 958
Cow2c10          ACGTGGAGCGTGCGGTTTAATTTGACTCAACACGGGGAACTTTACCAGATCCGGACAGGG 958
AB007814Tth      ACGTGGAGCGTGCGGTTTAATTTGACTCAACACGGGGAACTTTACCAGATCCGGACAGGG 958
Cow104c2         ACGTGGAGCGTGCGGTTTAATTTGACTCAACACGGGGAACTTTACCAGATCCGGACAGGG 958
Cow104c3         ACGTGGAGCGTGCGGTTTAATTTGACTCAACACGGGGAACTTTACCAGATCCGGACAGGG 958
Cow104c1         ACGTGGAGCGTGCGGTTTAATTTGACTCAACACGGGGAACTTTACCAGATCCGGACAGGG 958
Cow2095c4        ACGTGGAGCGTGCGGTTTAATTTGACTCAACACGGGGAACTTTACCAGATCCGGACAGGG 958
Cow2095c9        ACGTGGAGCGTGCGGTTTAATTTGACTCAACACGGGGAACTTTACCAGATCCGGACAGGG 958
Cow2095c8        ACGTGGAGCGTGCGGTTTAATTTGACTCAACACGGGGAACTTTACCAGATCCGGACAGGG 958
WTDA3c9.1        ACGTGGAGCGTGCGGTTTAATTTGACTCAACACGGGGAACTTTACCAGATCCGGACAGGG 956
WTDA3c9.4        ACGTGGAGCGTGCGGTTTAATTTGACTCAACACGGGGAACTTTACCAGATCCGGACAGGG 956
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Elk142c2         TGAGGATTGACAGATTGAGTGTTCTTTCTCGATCCCCTGAATGGTGGTGCATGGCCGCTT 1018
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|---|---|---|
| Elk142c10 | TGAGGATTGACAGATTGAGTGTTCTTTCTCGATCCCCTGAATGGTGGTGCATGGCCGCTT | 1018 |
| Elk142c5 | TGAGGATTGACAGATTGAGTGTTCTTTCTCGATCCCCTGAATGGTGGTGCATGGCCGCTT | 1017 |
| Elk328c3 | TGAGGATTGACAGATTGAGTGTTCTTTCTCGATCCCCTGAATGGTGGTGCATGGCCGCTT | 1018 |
| Elk416c8 | TGAGGATTGACAGATTGAGTGTTCTTTCTCGATCCCCTGAATGGTGGTGCATGGCCGCTT | 1018 |
| WTDN15c1 | TGAGGATTGACAGATTGAGTGTTCTTTCTCGATCCCCTGAATGGTGGTGCATGGCCGCTT | 1017 |
| WTDNL15c6 | TGAGGATTGACAGATTGAGTGTTCTTTCTCGATCCCCTGAATGGTGGTGCATGGCCGCTT | 1017 |
| WTDN15c9 | TGAGGATTGACAGATTGAGTGTTCTTTCTCGATCCCCTGAATGGTGGTGCATGGCCGCTT | 1017 |
| WTDA21c4 | TGAGGATTGACAGATTGAGTGTTCTTTCTCGATCCCCTGA**G**TGGTGGTGCATGGCCGCTT | 1018 |
| WTDA21c5 | TGAGGATTGACAGATTGAGTGTTCTTTCTCGATCCCCTGAATGGTGGTGCATGGCCGCTT | 1017 |
| WTDA21c6 | TGAGGATTGACAGATTGAGTGTTC**C**TTCTCGATCCCCTGAATGGTGGTGCATGGCCGCTT | 1016 |
| Cow139c10 | TGAGGATTGACAGATTGAGTGTTCTTTCTCGATCCCCTGAATGGTGGTGCATGGCCGCTT | 1018 |
| Cow139c11 | TGAGGATTGACAGATTGAGTGTTCTTTCTCGATCCCCTGAATGG**C**GGTGCATGGCCGCTT | 1018 |
| Cow2c4 | TGAGGATTGACAGATTGAGTGTTCTTTCTCGATCCCCTGAATGGTGGTGCATGGCCGCTT | 1018 |
| Cow133c1 | TGAGGATTGACAGATTGAGTGTTCTTTCTCGATCCCCTGAATGGTGGTGCATGGCCGCTT | 1018 |
| Cow139c9 | TGAGGATTGACAGATTGAGTGTTCTTTCTCGATCCCCTGAATGGTGGTGCATGGCCGCTT | 1018 |
| Cow3535c3 | TGAGGATTGACAGATTGAGTGTTCTTTCTCGATCCCCTGAATGGTGGTGCATGGCCGCTT | 1018 |
| Cow3535c6 | TGAGGATTGACAGATTGAGTGTTCTTTCTCGATCCCCTGAATGGTGGTGCATGGCCGCTT | 1018 |
| Cow2c7 | TGAGGATTGACAGATTGAGTGTTCTTTCTCGATCCCCTGAATGGTGGTGCATGGCCGCTT | 1018 |
| Cow2c10 | TGAGGATTGACAGATTGAGTGTTCTTTCTCGATCCCCTGAATGGTGGTGCATGGCCGCTT | 1018 |
| AB007814Tth | TGAGGATTGACAGATTGAGTGTTCTTTCTCGATCCCCTGAATGGTGGTGCATGGCCGCTT | 1018 |
| Cow104c2 | TGAGGATTGACAGATTGAGTGTTCTTTCTCGATCCCCTGAATGGTGGTGCATGGCCGCTT | 1018 |
| Cow104c3 | TGAGGATTGACAGATTGAGTGTTCTTTCTCGATCCCCTGAATGGTGGTGCATGGCCGCTT | 1018 |
| Cow104c1 | TGAGGATTGACAGATTGAGTGTTCTTTCTCGATCCCCTGAATGGTGGTGCATGGCCGCTT | 1018 |
| Cow2095c4 | TGAGGATTGACAGATTGAGTGTTCTTTCTCGATCCCCTGAATGGTGGTGCATGGCCGCTT | 1018 |
| Cow2095c9 | TGAGGATTGACAGATTGAGTGTTCTTTCTCGATCCCCTGAATGGTGGTGCATGGCCGCTT | 1018 |
| Cow2095c8 | TGAGGATTGACAGATTGAGTGTTCTTTCTCGATCCCCTGAATGGTGGTGCATGGCCGCTT | 1018 |
| WTDA3c9.1 | TGAGGATTGACAGATTGAGTGTTCTTTCTCGATCCCCTGAATGGTGGTGCATGGCCGCTT | 1016 |
| WTDA3c9.4 | TGAGGATTGACAGATTGAGTGTTCTTTCTCGATCCCCTGAATGGTGGTGCATGGCCGCTT | 1016 |
| | ************************ *************** *** *************** | |
| | | |
| Elk142c2 | TTGGTCGGTGGAGTGATTTGTTTGGTTGATTCCGTCAACGGACGAGATCCAAGCTGCCCA | 1078 |
| Elk142c10 | TTGGTCGGTGGAGTGATTTGTTTGGTTGATTCCGTCAACGGACGAGATCCAAGCTGCCCA | 1078 |
| Elk142c5 | TTGGTCGGTGGAGTGATTTGTTTGGTTGATTCCGTCAACGGACGAGATCCAAGCTGCCCA | 1077 |
| Elk328c3 | TTGGTCGGTGGAGTGATTTGTTTGGTTGATTCCGTCAACGGACGAGATCCAAGCTGCCCA | 1078 |
| Elk416c8 | TTGGTCGGTGGAGTGATTTGTTTGGTTGATTCCGTCAACGGACGAGATCCAAGCTGCCCA | 1078 |
| WTDN15c1 | TTGGTCGGTGGAGTGATTTGTTTGGTTGATTCCGTCAACGGACGAGATCCAAGCTGCCCA | 1077 |
| WTDNL15c6 | TTGGTCGGTGGAGTGATTTGTTTGGTTGATTCCGTCAACGGACGAGATCCAAGCTGCCCA | 1077 |
| WTDN15c9 | TTGGTCGGTGGAGTGATTTGTTTGGTTGATTCCGTCAACGGACGAGATCCAAGCTGCCCA | 1077 |
| WTDA21c4 | TTGGTCGGTGGAGTGATTTGTTTGGTTGATTCCGTCAACGGACGAGATCCAAGCTGCCCA | 1078 |
| WTDA21c5 | TTGGTCGGTGGAGTGATTTGTTTGGTTGATTCCGTCAACGGACGAGATCCAAGCTGCCCA | 1077 |
| WTDA21c6 | TTGGTCGGTGGAGTGATTTGTTTGGTTGATTCCGTCAACGGACGAGATCCAAGCTGCCCA | 1076 |
| Cow139c10 | TTGGTCGGTGGAGTGATTTGTTTGGTT**A**ATTCCGTCAACGGACGAGATCCAAGCTGCCCA | 1078 |

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Cow139c11         TTGGTCGGTGGAGTGATTTGTTTGGTTGATTCCGTCAACGGACGAGATCCAAGCTGCCCA 1078
Cow2c4            TTGGTCGGTGGAGTGATTTGTTTGGTTGATTCCGTCAACGGACGAGATCCAAGCTGCCCA 1078
Cow133c1          TTGGTCGGTGGAGTGATTTGTTTGGTTGATTCCGTCAACGGACGAGATCCAAGCTGCCCA 1078
Cow139c9          TTGGTCGGTGGAGTGATTTGTTTGGTTGATTCCGTCAACGGACGAGATCCAAGCTGCCCA 1078
Cow3535c3         TTGGTCGGTGGAGTGATTTGTTTGGTTGATTCCGTCAACGGACGAGATCCAAGCTGCCCA 1078
Cow3535c6         TTGGTCGGTGGAGTGATTTGTTTGGTTGATTCCGTCAACGGACGAGATCCAGGCTGCCCA 1078
Cow2c7            TTGGTCGGTGGAGTGATTTGTTTGGTTGATTCCGTCAACGGACGAGATCCAAGCTGCCCA 1078
Cow2c10           TTGGTCGGTGGAGTGATTTGTTTGGTTGATTCCGTCAACGGACGAGATCCAAGCTGCCCA 1078
AB007814Tth       TTGGTCGGTGGAGTGATTTGTTTGGTTGATTCCGTCAACGGACGAGATCCAAGCTGCCCA 1078
Cow104c2          TTGGTCGGTGGAGTGATTTGTCTGGTTGATTCCGTCAACGGACGAGATCCAAGCTGCCCA 1078
Cow104c3          TTGGTCGGTGGAGTGATTTGTCTGGTTGATTCCGTCAACGGACGAGATCCAAGCTGCCCA 1078
Cow104c1          TTGGTCGGTGGAGTGATTTGTCTGGTTGATTCCGTCAACGGACGAGATCCAAGCTGCCCA 1078
Cow2095c4         TTGGTCGGTGGAGTGATTTGTTTGGTTGATTCCGTCAACGGACGAGATCCAAGCTGCCCA 1078
Cow2095c9         TTGGTCGGTGGAGTGATTTGTTTGGTTGATTCCGTCAACGGACGAGATCCAAGCTGCCCA 1078
Cow2095c8         TTGGTCGGTGGAGTGATTTGTTTGGTTGATTCCGTCAACGGACGAGATCCAAGCTGCCCA 1078
WTDA3c9.1         TTGGTCGGTGGAGTGATTTGTTTGGTTGATTCCGTCAACGGACGAGATCCAAGCTGCCCA 1076
WTDA3c9.4         TTGGTCGGTGGAGTGATTTGTTTGGTTGATTCCGTCAACGGACGAGATCCAAGCTGCCCA 1076
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Elk142c2          GTAGGATTCAGAATTGCCCATAGGATAGCAATCCCCTCCGCGGGTTTTTCCCAAGGAGGG 1138
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Elk142c5          GTAGGATTCAGAATTGCCCATAGGATAGCAATCCCCTCCGCGGGTTTTTCCCAAGGAGGG 1137
Elk328c3          GTAGGATTCAGAATTGCCCATAGGATAGCAATCCCCTCCGCGGGTTTTTCCCAAGGAGGG 1138
Elk416c8          GTAGGATTCAGAATTGCCCATAGGATAGCAATCCCCTCCGCGGGTTTTTCCCAAGGAGGG 1138
WTDN15c1          GTAGGATTCAGAATTGCCCATAGGATAGCAATCCCCTCCGCGGGTTTTTCCCAAGGAGGG 1137
WTDNL15c6         GTAGGATTCAGAATTGCCCATAGGATAGCAATCCCCTCCGCGGGTTTTTCCCAAGGAGGG 1137
WTDN15c9          GTAGGATTCAGAATTGCCCATAGGATAGCAATCCCCTCCGCGGGTTTTTCCCAAGGAGGG 1137
WTDA21c4          GTAGGATTCAGAATTGCCCATAGGATAGCAATCCCCTCCGCGGGTTTTTCCCAAGGAGGG 1138
WTDA21c5          GTAGGATTCAGAATTGCCCATAGGATAGCAATCCCCTCCGCGGGTTTTTCCCAAGGAGGG 1137
WTDA21c6          GTAGGATTCAGAATTGCCCATAGGATAGCAATCCCCTCCGCGGGTTTTTCCCAAGGAGGG 1136
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Cow139c11         GTAGGATTCAGAATTGCCCATAGGATAGCAATCCCCTCCGCGGGTTTTTCCCAAGGAGGG 1138
Cow2c4            GTAGGATTCAGAATTGCCCATAGGATAGCAATCCCCTCCGCGGGTTTTTCCCAAGGAGGG 1138
Cow133c1          GTAGGATTCAGAATTGCCCATAGGATAGCAATCCCCTCCGCGGGTTTTTCCCAAGGAGGG 1138
Cow139c9          GTAGGATTCAGAATTGCCCATAGGATAGCAATCCCCTCCGCGGGTTTTTCCCAAGGAGGG 1138
Cow3535c3         GTAGGATTCAGAATTGCCCATAGGATAGCAATCCCCTCCGCGGGTTTTTCCCAAGGAGGG 1138
Cow3535c6         GTAGGATTCAGAATTGCCCATAGGATAGCAATCCCCTCCGCGGGTTTTTCCCAAGGAGGG 1138
Cow2c7            GTAGGATTCAGAATTGCCCATAGGATAGCAATCCCCTCCGCGGGTTTTTCCCAAGGAGGG 1138
Cow2c10           GTAGGATTCAGAATTGCCCATAGGATAGCAATCCCCTCCGCGGGTTTTTCCCAAGGAGGG 1138
AB007814Tth       GTAGGATTCAGAATTGCCCATAGGATAGCAATCCCCTCCGCGGGTTTTTCCCAAGGAGGG 1138
Cow104c2          GTAGGATTCAGAATTGCCCATAGGATAGCAATCCCCTCCGCGGGTTTTCCCCAAGGAGGG 1138
Cow104c3          GTAGGATTCAGAATTGCCCATAGGATAGCAATCCCCTCCGCGGGTTTTCCCCAAGGAGGG 1138
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Cow104c1 GTAGGATTCAGAATTGCCCATAGGATAGCAATCCCCTCCGCGGGTTTTTCCCAAGGAGGG 1138
Cow2095c4 GTAGGATTCAGAATTGCCCATAGGATAGCAATCCCCTCCGCGGGTTTTTCCCAAGGAGGG 1138
Cow2095c9 GTAGGATTCAGAATTGCCCATAGGATAGCAATCCCCTCCGCGGGTTTTTCCCAAGGAGGG 1138
Cow2095c8 GTAGGATTCAGAATTGCCCATAGGATAGCAATCCCCTCCGCGGGTTTTTCCCAAGGAGGG 1138
WTDA3c9.1 GTAGGATTCAGAATTGCCCATAGGATAGCAATCCCCTCCGCGGGTTTTTCCCAAGGAGGG 1136
WTDA3c9.4 GTAGGATTCAGAATTGCCCATAGGATAGCAATCCCCTCCGCGGGTTTTTCCCAAGGAGGG 1136
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Elk142c10 GCGATATTCGTTTGTATCCTTCTCTGCGGGATTCCTTGTTTTGCGCAAGGTGAGATTTTG 1198
Elk142c5 GCGATATTCGTTTGTATCCTTCTCTGCGGGATTCCTTGTTTTGCGCAAGGTGAGATTTTG 1197
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WTDA21c5 GCGATATTCGTTTGTATCCTTCTCTGCGGGATTCCTTGTTTTGCGCAAGGTGAGATTTTG 1197
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Cow139c10 GCGATATTCGTTTGTATCCTTCTCTGCGGGATTCCTTGTTTTGCGCAAGGTGAGATTTTG 1198
Cow139c11 GCGATATTCGTTTGTATCCTTCTCTGCGGGATTCCTTGTTTTGCGCAAGGTGAGATTTTG 1198
Cow2c4 GCGATATTCGTTTGTATCCTTCTCTGCGGGATTCCTTGTTTTGCGCAAGGTGAGATTTTG 1198
Cow133c1 GCGATATTCGTTTGTATCCTTCTCTGCGGGATTCCTTGTTTTGCGCAAGGTGAGATTTTG 1198
Cow139c9 GCGATATTCGTTTGTATCCTTCTCTGCGGGATTCCTTGTTTTGCGCAAGGTGAGATTTTG 1198
Cow3535c3 GCGATATTCGTTTGTATCCTTCTCTGCGGGATTCCTTGTTTTGCGCAAGGTG**G**GATTTTG 1198
Cow3535c6 GCGATATTCGTTTGTATCCTTCTCTGCGGGATTCCTTGTTTTGCGCAAGGTGAGATTTTG 1198
Cow2c7 GCGATATTCGTTTGTATCCTTCTCTGCGGGATTCCTTGTTTTGCGCAAGGTGAGATTTTG 1198
Cow2c10 GCGATATTCGTTTGTATCCTTCTCTGCGGGATTCCTTGTTTTGCGCAAGGTGAGATTTTG 1198
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Cow104c2 GCGATATTCGTTTGTATCCTTCTCTGCGGGATTCCTTGTTTTGCGCAAGGTGAGATTTTG 1198
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Cow2095c4 GCGATATTCGTTTGTATCCTTCTCTGCGGGATTCCTTGTTTTGCGCAAGGTGAGATTTTG 1198
Cow2095c9 GCGATATTCGTTTGTATCCTTCTCTGCGGGATTCCTTGTTTTGCGCAAGGTGAGATTTTG 1198
Cow2095c8 GCGATATTCGTTTGTATCCTTCTCTGCGGGATTCCTTGTTTTGCGCAAGGTGAGATTTTG 1198
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WTDA3c9.4 GCGATATTCGTTTGTATCCTTCTCTGCGGGATTCCTTGTTTTGCGCAAGGTGAGATTTTG 1196
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Elk142c10 GGCAACAGCAGGTC**C**GTGATGCTCCTCAATGTTCTGGGCGACACGCGCACTACAATGTCA 1258

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Elk142c5          GGCAACAGCAGGTCTGTGATGCTCCTCAATGTTCTGGGCGGCACGCGCACTACAATGTCA 1257
Elk328c3          GGCAACAGCAGGTCTGTGATGCTCCTCAATGTTCTGGGCGACACGCGCACTACAATGTCA 1258
Elk416c8          GGCAACAGCAGGTCTGTGATGCTCCTCAATGTTCTGGGCGACACGCGCACTACAATGTCA 1258
WTDN15c1          GGCAACAGCAGGTCTGTGATGCTCCTCAATGTTCTGGGCGACACGCGCACTACAATGTCA 1257
WTDNL15c6         GGCAACAGCAGGTCTGTGATGCTCCTCAATGTTCTGGGCGACACGCGCACTACAATGTCA 1257
WTDN15c9          GGCAACAGCAGGTCTGTGATGCTCCTCAATGTTCTGGGCGACACGCGCACTACAATGTCA 1257
WTDA21c4          GGCAACAGCAGGTCTGTGATGCTCCTCAATGTTCTGGGCGACACGCGCACTACAATGTCA 1258
WTDA21c5          GGCAACAGCAGGTCTGTGATGCTCCTCAATGTTCTGGGCGACACGCGCACTACAATGTCA 1257
WTDA21c6          GGCAACAGCAGGTCTGTGATGCTCCTCAATGTTCTGGGCGACACGCGCACTACAATGTCA 1256
Cow139c10         GGCAACAGCAGGTCTGTGATGCTCCTCAATGTTCTGGGCGACACGCGCACTACAATGTCA 1258
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Cow2c4            GGCAACAGCAGGTCTGTGATGCTCCTCAATGTTCTGGGCGACACGCGCACTACAATGTCA 1258
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Cow104c1          GGCAACAGCAGGTCTGTGATGCTCCTCAATGTTCTGGGCGACACGCGCACTACAATGTCA 1258
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Cow2095c9         GGCAACAGCAGGTCTGTGATGCTCCTCAATGTTCTGGGCGACACGCGCACTACAATGTCA 1258
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WTDA3c9.4         GGCAACAGCAGGTCTGTGATGCTCCTCAATGTTCTGGGCGACACGCGCACTACAATGTCA 1256
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Elk142c2          GTGAGAACAAGAAAAACGACTTTTGTCGGACCTACTTGATCAAAAGAGTGGGAAAACCCC 1318
Elk142c10         GTGAGAACAAGAAAAACGACTTTTGTCGGACCTACTTAATCAAAAGAGTGGGAAAACCCC 1318
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Elk328c3          GTGAGAACAAGAAAAACGACTTTTGTCGGACCTACTTGATCAAAAGAGTGGGAAAACCCC 1318
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Cow3535c3       GTGAGAACAAGAAAAACGACTTTTGTCGGACCTACTTGATCAAAAGGGTGGGAAAACCCC 1318
Cow3535c6       GTGAGAACAAGAAAAACGACTTTTGTCGGACCTACTTGATCAAAAGGGTGGGAAAACCCC 1318
Cow2c7          GTGAGAACAAGAAAAACGACTTTTGTCGGACCTACTTGATCAAAAGAGTGGGAAAACCCC 1318
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AB007814Tth     GTGAGAACAAGAAAAACGACTTTTGTCGGACCTACTTGATCAAAAGAGTGGGAAAACCCC 1318
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Cow2095c4       GTGAGAACAAGAAAAACGACTTTTGTCGGACCTACTTGATCAAAAGAGTGGGAAAACCCC 1318
Cow2095c9       GTGAGAACAAGAAAAACGACTTTTGTCGGACCTACTTGATCAAAAGAGTGGGAAAACCCC 1318
Cow2095c8       GTGAGAACAAGAAAAACGACTTTTGTCGGACCTACTTGATCAAAAGAGTGGGAAAACCCC 1318
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WTDA3c9.4       GTGAGAACAAGAAAAACGACTTTTGTCGGACCTACTTGATCAAAAGAGTGGGAAAACCCC 1316
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Elk142c2        GGAATCACATAGACCCACTTGGGACCGAGTATTGCAATTATCGGTCGCGCAACGAGGAAT 1378
Elk142c10       GGAATCACATAGACCCACTTGGGACCGAGTATTGCAATTATTGGTCGCGCAACGAGGAAT 1378
Elk142c5        GGAATCACATAGACCCACTTGGGACCGAGTATTGCAATTATTGGTCGCGCAACGAGGAAT 1377
Elk328c3        GGAATCACATAGACCCACTTGGGACCGAGTATTGCAATTATTGGTCGCGCAACGAGGAAT 1378
Elk416c8        GGAATCACATAGACCCACTTGGGACCGAGTATTGCAATTATTGGTCGCGCAACGAGGAAT 1378
WTDN15c1        GGAATCACATAGACCCACTTGGGACCGAGTATTGCAATTATTGGTCGCGCAACGAGGAAT 1377
WTDNL15c6       GGAATCACATAGACCCACTTGGGACCGAGTATTGCAATTATCGGTCGCGCAACGAGGAAT 1377
WTDN15c9        GGAATCACATAGACCCACTTGGGACCGAGTATTGCAATTATTGGTCGCGCAACGAGGAAT 1377
WTDA21c4        GGAATCACATAGACCCACTTGGGACCGAGTATTGCAATTATTGGTCGCGCAACGAGGAAT 1378
WTDA21c5        GGAATCACATAGACCCACTTGGGACCGAGTATTGCAATTATTGGTCGCGCAACGAGGAAT 1377
WTDA21c6        GGAATCACATAGACCCACTTGGGACCGAGTATTGCAATTATTGGTCGCGCAACGAGGAAT 1376
Cow139c10       GGAATCACATAGACCCACTTGGGACCGAGTATTGCAATTATTGGTCGCGCAACGAGGAAT 1378
Cow139c11       GGAATCACATAGACCCACTTGGGACCGAGTATTGCAATTATTGGTCGCGCAACGAGGAAT 1378
Cow2c4          GGAATCACATAGACCCACTTGGGACCGAGTATTGCAATTATTGGTCGCGCAACGAGGAAT 1378
Cow133c1        GGAATCACATAGACCCACTTGGGACCGAGTATTGCAATTATTGGTCGCGCAACGAGGAAT 1378
Cow139c9        GGAATCGCATAGACCCACTTGGGACCGAGTATTGCAATTATTGGTCGCGCAACGAGGAAT 1378
Cow3535c3       GGAATCACATAGACCCACTTGGGACCGAGTATTGCAATTATTGGTCGCGCAACGAGGAAT 1378
Cow3535c6       GGAATCACATAGACCCACTTGGGACCGAGTATTGCAATTATTGGTCGCGCAACGAGGAAT 1378
Cow2c7          GGAATCACATAGACCCACTTGGGACCGAGTATTGCAATTATTGGTCGCGCAACGAGGAAT 1378
Cow2c10         GGAATCACATAGACCCACTTGGGACCGAGTATTGCAATTATTGGTCGCGCAACGAGGAAT 1378
AB007814Tth     GGAATCACATAGACCCACTTGGGACCGAGTATTGCAATTATTGGTCGCGCAACGAGGAAT 1378
Cow104c2        GGAATCACATAGACCCACTTGGGACCGAGTATTGCAATTATTGGTCGCGCAACGAGGAAT 1378
Cow104c3        GGAATCACATAGACCCACTTGGGACCGAGTATTGCAATTATTGGTCGCGCAACGAGGAAT 1378
Cow104c1        GGAATCACATAGACCCACTTGGGACCGAGTATTGCAATTATTGGTCGCGCAACGAGGAAT 1378
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Cow2095c4       GGAATCACATAGACCCACTTGGGACCGAGTATTGCAATTATTGGTCGCGCAACGAGGAAT 1378
Cow2095c9       GGAATCACATAGACCCACTTGGGACCGAGTATTGCAATTATTGGTCGCGCAACGAGGAAT 1378
Cow2095c8       GGAATCACATAGACCCACTTGGGACCGAGTATTGCAATTATTGGTCGCGCAACGAGGAAT 1378
WTDA3c9.1       GGAATCACATAGACCCACTTGGGACCGAGTATTGCAATTATTGGTCGCGCAACGAGGAAT 1376
WTDA3c9.4       GGAATCACATAGACCCACTTGGGACCGAGTATTGCAATTATTGGTCGCGCAACGAGGAAT 1376
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Elk142c2        GTCTCGTAGGCGCAGCTCATCAAACTGTGCCGATTACGTCCCTGCCATTTGTACACACCG 1438
Elk142c10       GTCTCGTAGGCGCAGCTCATCAAACTGTGCCGATTACGTCCCTGCCATTTGTACACACCG 1438
Elk142c5        GTCTCGTAGGCGCAGCTCATCAAACTGTGCCGATTACGTCCCTGCCATTTGTACACACCG 1437
Elk328c3        GTCTCGTAGGCGCAGCTCATCAAACTGTGCCGATTACGTCCCTGCCATTTGTACACACCG 1438
Elk416c8        GTCTCGTAGGCGCAGCTCATCAAACTGTGCCGATTACGTCCCTGCCATTTGTACACACCG 1438
WTDN15c1        GTCTCGTAGGCGCAGCTCATCAAACTGTGCCGATTACGTCCCTGCCATTTGTACACACCG 1437
WTDNL15c6       GTCTCGTAGGCGCAGCTCATCAAACTGTGCCGATTACGTCCCTACCATTTGTACACACCG 1437
WTDN15c9        GTCTCGTAGGCGCAGCTCATCAAACTGTGCCGATTACGTCCCTGCCATTTGTACACACCG 1437
WTDA21c4        GTCTCGTAGGCGCAGCTCATCAAACTGTGCCGATTACGTCCCTGCCATTTGTACACACCG 1438
WTDA21c5        GTCTCGTAGGCGCAGCTCATCAAACTGTGCCGATTACGTCCCTGCCATTTGTACACACCG 1437
WTDA21c6        GTCTCGTAGGCGCAGCTCATCAAACTGTGCCGATTACGTCCCTGCCATTTGTACACACCG 1436
Cow139c10       GTCTCGTAGGCGCAGCTCATCAAACTGTGCCGATTACGTCCCTGCCATTTGTACACACCG 1438
Cow139c11       GTCTCGTAGGCGCAGCTCATCAAACTGTGCCGATTACGTCCCTGCCATTTGTACACACCG 1438
Cow2c4          GTCTCGTAGGCGCAGCTCATCAAACTGTGCCGATTACGTCCCTGCCATTTGTACACACCG 1438
Cow133c1        GTCTCGTAGGCGCAGCTCATCAAACTGTGCCGATTACGTCCCTGCCATTTGTACACACCG 1438
Cow139c9        GTCTCGTAGGCGCAGCTCATCAAACTGTGCCGATTACGTCCCTGCCATTTGTACACACCG 1438
Cow3535c3       GTCTCGTAGGCGCAGCTCATCAAACTGTGCCGATTACGTCCCTGCCATTTGTACACACCG 1438
Cow3535c6       GTCTCGTAGGCGCAGCTCATCAAACTGTGCCGATTACGTCCCTGCCATTTGTACACACCG 1438
Cow2c7          GTCTCGTAGGCGCAGCTCATCAAACTGTGCCGATTACGTCCCTGCCATTTGTACACACCG 1438
Cow2c10         GTCTCGTAGGCGCAGCTCATCAAACTGTGCCGATTACGTCCCTGCCATTTGTACACACCG 1438
AB007814Tth     GTCTCGTAGGCGCAGCTCATCAAACTGTGCCGATTACGTCCCTGCCATTTGTACACACCG 1438
Cow104c2        GTCTCGTAGGCGCAGCTCATCAAACTGTGCCGATTACGTCCCTGCCATTTGTACACACCG 1438
Cow104c3        GTCTCGTAGGCGCAGCTCATCAAACTGTGCCGATTACGTCCCTGCCATTTGTACACACCG 1438
Cow104c1        GTCTCGTAGGCGCAGCTCATCAAACTGTGCCGATTACGTCCCTGCCATTTGTACACACCG 1438
Cow2095c4       GTCTCGTAGGCGCAGCTCATCAAACTGTGCCGATTACGTCCCTGCCATTTGTACACACCG 1438
Cow2095c9       GTCTCGTAGGCGCAGCTCATCAAACTGTGCCGATTACGTCCCTGCCATTTGTACACACCG 1438
Cow2095c8       GTCTCGTAGGCGCAGCTCATCAAACTGTGCCGATTACGTCCCTGCCATTTGTACACACCG 1438
WTDA3c9.1       GTCTCGTAGGCGCAGCTCATCAAACTGTGCCGATTACGTCCCTGCCATTTGTACACACCG 1436
WTDA3c9.4       GTCTCGTAGGCGCAGCTCATCAAACTGTGCCGATTACGTCCCTGCCATTTGTACACACCG 1436
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Elk142c2        CCCGTCGTTGTTTCCGATGATGGTGCAATACAGGTGAACGGACAGTCGAACGTTTCGTTT 1498
Elk142c10       CCCGTCGTTGTTTCCGATGATGGTGCAACACAGGTGAACGGACAGTCGAACGTTTCGTTT 1498
Elk142c5        CCCGTCGTTGTTTCCGATGATGGTGCAATACAGGTGAACGGACAGTCGAACGTTTCGTTT 1497
Elk328c3        CCCGTCGTTGTTTCCGATGATGGTGCAATACAGGTGAACGGACAGTCGAACGTTTCGTTT 1498
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Elk416c8         CCCGTCGTTGTTTCCGATGATGGTGCAATACAGGTGAACGGACAGTCGAACGTTTCGTTT 1498
WTDN15c1         CCCGTCGTTGTTTCCGATGATGGTGCAATACAGGTGAACGGACAGTCGAACGTTTCGTTT 1497
WTDNL15c6        CCCGTCGTTGTTTCCGATGATGGTGCAATACAGGTGAACGGACAGTCGAACGTTTCGTTT 1497
WTDN15c9         CCCGTCGTTGTTTCCGATGATGGTGCAATACAGGTGAACGGACAGTCGAACGTTTCGTTT 1497
WTDA21c4         CCCGTCGTTGTTTCCGATGATGGTGCAATACAGGTGAACGGACAGTCGAACGTTTCGTTT 1498
WTDA21c5         CCCGTCGTTGTTTCCGATGATGGTGCAATACAGGTGAACGGACAGTCGAGCGTTTCGTTT 1497
WTDA21c6         CCCGTCGTTGTTTCCGATGATGGTGCAATACAGGTGAACGGACAGTCGAACGTTTCGTTT 1496
Cow139c10        CCCGTCGTTGTTTCCGATGATGGTGCAATACAGGTGAACGGACAGTCGAACGTTTCGTTT 1498
Cow139c11        CCCGTCGTTGTTTCCGATGATGGTGCAATACAGGCGAACGGACAGTCGAACGTTTCGTTT 1498
Cow2c4           CCCGTCGTTGTTTCCGATGATGGTGCAATACGGGTGAACGGACAGTCGAACGTTTCGTTT 1498
Cow133c1         CCCGTCGTTGTTTCCGATGATGGTGCAATACAGGTGAACGGACAGTCGAACGTTTCGTTT 1498
Cow139c9         CCCGTCGTTGTTTCCGATGATGGTGCAATACAGGTGAACGGACAGTCGAACGTTTCGTTT 1498
Cow3535c3        CCCGTCGTTGTTTCCGATGATGGTGCAATACAGGTGAACGGACAGTCGAACGTTTCGTTT 1498
Cow3535c6        CCCGTCGTTGTTTCCGATGATGGTGCAATACAGGTGAACGGACAGTCGAACGTTTCGTTT 1498
Cow2c7           CCCGTCGTTGTTTCCGATGATGGTGCAATACAGGTGAACGGACAGTCGAACGTTTCGTTT 1498
Cow2c10          CCCGTCGTTGTTTCCGATGATGGTGCAATACAGGTGAACGGACAGTCGAACGTTTCGTTT 1498
AB007814Tth      CCCGTCGTTGTTTCCGATGATGGTGCAATACAGGTGAACGGACAGTCGAACGTTTCGTTT 1498
Cow104c2         CCCGTCGTTGTTTCCGATGATGGTGCAATACAGGTGAACGGACAGTCGAACGTTTCGTTT 1498
Cow104c3         CCCGTCGTTGTTTCCGATGATGGTGCAATACAGGTGAACGGACAGTCGAACGTTTCGTTT 1498
Cow104c1         CCCGTCGTTGTTTCCGATGATGGTGCAATACAGGTGAACGGACAGTCGAACGTTTCGTTT 1498
Cow2095c4        CCCGTCGTTGTTTCCGATGATGGTGCAATACAGGTGAACGGACAGTCGAACGTTTCGTTT 1498
Cow2095c9        CCCGTCGTTGTTTCCGATGATGGTGCAATACAGGTGAACGGACAGTCGAACGTTTCGTTT 1498
Cow2095c8        CCCGTCGTTGTTTCCGATGATGGTGCAATACAGGTGAACGGACAGTCGAACGTTTCGTTT 1498
WTDA3c9.1        CCCGTCGTTGTTTCCGATGATGGTGCAATACAGGTGAACGGACAGTCGAACGTTTCGTTT 1496
WTDA3c9.4        CCCGTCGTTGTTTCCGATGATGGTGCAATACAGGTGAACGGACAGTCGAACGTTTCGTTT 1496
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Elk142c2         GACCGAAAGTTCACCGATATTTCTTCAATAGAGGAAGCAAAAGTCGTAACAAGGTAGCTG 1558
Elk142c10        GACCGAAAGTTCACCGATATTTCTTCAATAGAGGAAGCAAAAGTCGTAACAAGGTAGCTG 1558
Elk142c5         GACCGAAAGTTCACCGATATTTCTTCAATAGAGGAAGCAAAAGTCGTAACAAGGTAGCTG 1557
Elk328c3         GACCGAAAGTTCACCGATATTTCTTCAATAGAGGAAGCAAAAGTCGTAACAAGGTAGCTG 1558
Elk416c8         GACCGAAAGTTCACCGATATTTCTTCAATAGAGGAAGCAAAAGTCGTAACAAGGTAGCTG 1558
WTDN15c1         GACCGAAAGTTCACCGATATTTCTTCAATAGAGGAAGCAAAAGTCGTAACAAGGTAGCTG 1557
WTDNL15c6        GACCGAAAGTTCACCGATATTTCTTCAATAGAGGAAGCAAAAGTCGTAACAAGGTAGCTG 1557
WTDN15c9         GACCGAAAGTTCACCGATATTTCTTCAATAGAGGAAGCAAAAGTCGTAACAAGGTAGCTG 1557
WTDA21c4         GACCGAAAGTTCACCGATATTTCTTCAATAGAGGAAGCAAAAGTCGTAACAAGGTAGCTG 1558
WTDA21c5         GACCGAAAGTTCACCGATATTTCTTCAATAGAGGAAGCAAAAGTCGTAACAAGGTAGCTG 1557
WTDA21c6         GACCGAAAGTTCACCGATATTTCTTCAATAGAGGAAGCAAAAGTCGTAACAAGGTAGCTG 1556
Cow139c10        GACCGAAAGTTCACCGATATTTCTTCAATAGAGGAAGCAAAAGTCGTAACAAGGTAGCTG 1558
Cow139c11        GACCGAAAGTTCACCGATATTTCTTCAATAGAGGAAGCAAAAGTCGTAACAAGGTAGCTG 1558
Cow2c4           GACCGAAAGTTCACCGATATTTCTTCAATAGAGGAAGCAAAAGTCGTAACAAGGTAGCTG 1558
Cow133c1         GACTGAAAGTTCACCGATATTTCTTCAATAGAGGAAGCAAAAGTCGTAACAAGGTAGCTG 1558
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□Cow139c9        GACCGAAAGTTCACCGATATTTCTTCAATAGAGGAAGCAAAAGTCGTAACAAGGTAGCTG 1558
□Cow3535c3       GACCGAAAGTTCACCGATATTTCTTCAATAGAGGAAGCAAAAGTCGTAACAAGGTAGCTG 1558
□Cow3535c6       GACCGAAAGTTCACCGATATTTCTTCAATAGAGGAAGCAAAAGTCGTAACAAGGTAGCTG 1558
□Cow2c7          GACCGAAAGTTCACCGATATTTCTTCAATAGAGGAAGCAAAAGTCGTAACAAGGTAGCTG 1558
□Cow2c10         GACCGAAAGTTCACCGATATTTCTTCAATAGAGGAAGCAAAAGTCGTAACAAGGTAGCTG 1558
□AB007814Tth     GACCGAAAGTTCACCGATATTTCTTCAATAGAGGAAGCAAAAGTCGTAACAAGGTAGCTG 1558
□Cow104c2        GACCGAAAGTTCACCGATATTTCTTCAATAGAGGAAGCAAAAGTCGTAACAAGGTAGCTG 1558
□Cow104c3        GACCGAAAGTTCACCGATATTTCTTCAATAGAGGAAGCAAAAGTCGTAACAAGGTAGCTG 1558
□Cow104c1        GACCGAAAGTTCACCGATATTTCTTCAATAGAGGAAGCAAAAGTCGTAACAAGGTAGCTG 1558
□Cow2095c4       GACCGAAAGTTCACCGATATTTCTTCAATAGAGGAAGCAAAAGTCGTAACAAGGTAGCTG 1558
□Cow2095c9       GACCGAAAGTTCACCGATATTTCTTCAATAGAGGAAGCAAAAGTCGTAACAAGGTAGCTG 1558
□Cow2095c8       GACCGAAAGTTCACCGATATTTCTTCAATAGAGGAAGCAAAAGTCGTAACAAGGTAGCTG 1558
□WTDA3c9.1       GACCGAAAGCTCACCGATATTTCTTCAATAGAGGAAGCAAAAGTCGTAACAAGGTAGCTG 1556
□WTDA3c9.4       GACCGAAAGCTCACCGATATTTCTTCAATAGAGGAAGCAAAAGTCGTAACAAGGTAGCTG 1556
□                *** ***** **************************************************
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□Elk142c2        TAGGTGAACCTGCAGCTGGATCATTTTCCGATGAGATTATGTATCACACATATTT----- 1613
□Elk142c10       TAGGTGAACGTGCAGCTGGATCATTTTCCGATGAGATTATGTATCACACATATTTTT--- 1615
□Elk142c5        TAGGTGAACCTGCAGCTGGATCATTTTCCGATGAGATTATGTATCACACATATTT----- 1612
□Elk328c3        TAGGTGAACCTGCAGCTGGATCATTTTCCGATGAGATTATGTATCACACATATATTTA-- 1616
□Elk416c8        TAGGTGAACCTGCAGCTGGATCATTTTCCGATGAGATTATGTATCACACATATATTTA-- 1616
□WTDN15c1        TAGGTGAACCTGCAGCTGGATCATTTTCCGATGAGATTATGTATCACACATATATTTATT 1617
□WTDNL15c6       TAGGTGAACCTGCAGCTGGATCATTTTCCGATGAGATTATGTATCACACATATATTTA-- 1615
□WTDN15c9        TAGGTGAACCTGCAGCTGGATCATTTTCCGATGAGATTATGTATCACACATATATTTA-- 1615
□WTDA21c4        TAGGTGAACCTGCAGCTGGATCATTTTCCGATGAGATTATGTATCACACATATATTTATT 1618
□WTDA21c5        TAGGTGAACCTGCAGCTGGATCATTTTCCGATGAGATTATGTATCACACATATATTTATT 1617
□WTDA21c6        TAGGTGAACCTGCAGCTAGATCATTTTCCGATGAGATTATGTATCACACATATATTTAT- 1615
□Cow139c10       TAGGTGAACCTGCAGCTGGATCATTTTCCGATGAGATTATGTATCACACATATTTTTA-- 1616
□Cow139c11       TAGGTGAACCTGCAGCTGGATCATTTTCCGATGAGATTATGTATCACACATATTTTTA-- 1616
□Cow2c4          TAGGTGAACCTGCAGCTGGATCATTTTCCGATGAGATTATGTATCACACATATTTTTA-- 1616
□Cow133c1        TAGGTGAACCTGCAGCCGGATCATTTTCCGATGAGATTATGTATCACACATATTTTTA-- 1616
□Cow139c9        TAGGTGAATCTGCAGCTGGATCATTTTCCGATGAGATTATGTATCACACATATTTTTA-- 1616
□Cow3535c3       TAGGTGAACCTGCAGCTGGATCATTTTCCGATGAGATTATGTATCACACATATTTTTA-- 1616
□Cow3535c6       TAGGTGAACCTGCAGCTGGATCATTTTCCGATGAGATTATGTATCACACATATTTTTA-- 1616
□Cow2c7          TAGGTGAACCTGCAGCTGGATCATTTTCCGATGAGATTATGTATCACACATATTTTTA-- 1616
□Cow2c10         TAGGTGAACCTGCAGCTGGATCATTTTCCGATGAGATTATGTATCACACATATTTTTA-- 1616
□AB007814Tth     TAGGTGAACCTGCAGCTGGATCATTTTCCGATGAGATTATGTATCACACATATTTTTA-- 1616
□Cow104c2        TAGGTGAACCTGCAGCTGGATCATTTTCCGATGAGATTATGTATCACACATATTTAT--- 1615
□Cow104c3        TAGGTGAACCTGCAGCTGGATCATTTTCCGATGAGATTATGTATCACACATATTTAT--- 1615
□Cow104c1        TAGGTGAACCTGCAGCTGGATCATTTTCCGATGAGATTATGTATCACACATATTTAT--- 1615
□Cow2095c4       TAGGTGAACCTGCAGCTGGATCATTTTCCGATGAGATTATGTATCACACATATTTAT--- 1615
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Cow2095c9        TAGGTGAACCTGCAGCTGGATCATTTTCCGATGAGATTATGTATCACACATATTTAT--- 1615
Cow2095c8        TAGGTGAACCTGCAGCTGGATCATTTTCCGATGAGATTATGTATCACACATATTTAT--- 1615
WTDA3c9.1        TAGGTGAACCTGCAGCTGGATCATTTTCCGATGAGATTATGTATCACAAACACATGTA-- 1614
WTDA3c9.4        TAGGTGAACCTGCAGCTGGATCATTTTCCGATGAGATTATGTATCACAAACACATGTA-- 1614
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Elk142c2         --ATATGTACCGCGGGGTGGAA--TAGTAT---TTATGTATATATTATTATATACAT-AT 1665
Elk142c10        --ATATGTACCGCGGGGTGGAA--TAGTAT---TTATGTATATATTATTACATACAT-AA 1667
Elk142c5         --ATATGTACCGCGGGGTGGAA--TA-------------------------TACATAT-AT 1643
Elk328c3         -TATATGTACCGCGGGGTGGAA--TAGTAT---T-GTGTATAT----TTATATACAT-AT 1664
Elk416c8         -TATATGTACCGCGGGGTGGAA--TAGTAT---T-GTGTATAT----TTATATACAT-AT 1664
WTDN15c1         ATATATGTACCGCGGGGTGGAAAATA-TAT---T-GTGTATAT----TTATATGCAT-AT 1667
WTDNL15c6        -TATATGTACCGCGGGGTGGAAAATA-TAT---T-GTGTATAT----TTATATGCAT-AT 1664
WTDN15c9         -TATATGTACCGCGGGGTGGAAAATA-TAT---T-GTGTATAT----TTATATGCAT-AT 1664
WTDA21c4         ATATATGTACCGCGGGGTGGAAAATAGTAT---T-GTGTATAT----TTATATGCAT-AT 1669
WTDA21c5         ATATATGTACCGCGGGGTGGAAAATAGTAT---T-GTGTATAT----TTATATGCAT-AT 1668
WTDA21c6         ATATATGTACCGCGGGGTGGAA--TAATAT---T-GTGTATAT----TTATATACAT-AT 1664
Cow139c10        ---TATGTACCGCGGGGTGGAA--TAATATATTTTATGTATGTA----TATAT--AC-AT 1664
Cow139c11        ---TATGTACCGCGGGGTGGAA--TAATATATTTTATGTATGTA----TATAT--AC-AT 1664
Cow2c4           ---TATGTACCGCGGGGTGGAA--TAATATATTTTATGTATGTA----TATAT--AC-AT 1664
Cow133c1         ---TATGTACCGCGGGGTGGAA--TAATATATTTTATGTATGTA----TATAT--AC-AT 1664
Cow139c9         ---TATGTACCGCGGGGTGGAA--TAATATATTTTATGTATGTA----TATAT--AC-AT 1664
Cow3535c3        ---TATGTACCGCGGGGTGGAA--TAATATATTTTATGTATGTA----TATAT--AC-AT 1664
Cow3535c6        ---TATGTACCGCGGGGTGGAA--TAATATATTTTATGTATGTA----TATAT--AC-AT 1664
Cow2c7           ---TATGTACCGCGGGGTGGAA--TAATATATTTTATGTATGTA----TATAT--AC-AT 1664
Cow2c10          ---TATGTACCGCGGGGTGGAA--TAATATATTTTATGTATGTA----TATAT--AC-AT 1664
AB007814Tth      ---TATGTACCGCGGGGTGGAA--TAATATATTTTATGTATGTA----TATAT--AC-AT 1664
Cow104c2         ---TATGTACCGCGGGGTGGAA--TAATATATTTTATGTATGTA----TATATGTAC-AT 1665
Cow104c3         ---TATGTACCGCGGGGTGGAA--TAATATATTTTATGTATGTA----TATATGTAC-AT 1665
Cow104c1         ---TATGTACCGCGGGGTGGAA--TAATATATTTTATGTATGTA----TATATGTAC-AT 1665
Cow2095c4        ---TATGTACCGCGGGGTGGAA--TAATATATTTTATGTATATG----TATATATAC-AT 1665
Cow2095c9        ---TATGTACCGCGGGGTGGAA--TAATATATTTTATGTATATG----TATATATAC-AT 1665
Cow2095c8        ---TATGTACCGCGGGGTGGAA--TAATATATTTTATGTATATG----TATATATAC-AT 1665
WTDA3c9.1        ---TAT-----------TATAA--TAATATG---CATGTGTAC-----CGCGGGGAGGAA 1650
WTDA3c9.4        ---TAT-----------TATAA--TAATATG---CATGTGTAC-----CGCGGGGAGGAA 1650
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Elk142c2         ATATGTA--TTTCCTCCTTCGCACAGATATAACACATATTGCATTT-CGTCTGTTGC--T 1720
Elk142c10        ATATGTA--TTTCCTCCTTCGCACAGATATAACACATATTGCATTTTCGTCTGTTGC--T 1723
Elk142c5         ATATGTA--TTTCCTCCTTCGCACAGATATAACACATATTGCATTTTCGTCTGTTGC--T 1699
Elk328c3         ATATATATATTTCCTCCTTCGCACAGATATATTACATATTGCATTT-TGTCTGTTGTGTT 1723
Elk416c8         ATATATATATTTCCTCCTTCGCACAGATATATTACATATTGCATTT-TGTCTGTTGTGTT 1723
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WTDN15c1        ATATATATATTTCCTCCTTCGCACAGATATATTACATATTGCATTT-TGTCTGTTGTGTT 1726
WTDNL15c6       ATATATA-ATTTCCTCCTTCGCACAGATATATTACATATTGCATTT-TGTCTGTTGTGTT 1722
WTDN15c9        ATATATA--TTTCCTCCTTCGCACAGATATATTACATATTGCATTT-TGTCTGTTGTGTT 1721
WTDA21c4        ATATATA--TTTCCTCCTTCGCACAGATATATTACATATTGCATTT-TGTCTGTTGTGTT 1726
WTDA21c5        ATATATA--TTTCCTCCTTCGCACAGATATATTACATATTGCATTT-TGTCTGTTGTGTT 1725
WTDA21c6        ATATATG--TTTCCTCCTTCGCACAGATATATTACATATTGCATTT-TGTCTGTTGTGTT 1721
Cow139c10       ATGTATA--TTTCCTCCTTCGCACAGATGTATTACATATTGCATTT-TGTCTGTTGT--T 1719
Cow139c11       ATGTATA--TTTCCTCCTTCGCACAGATGTATTACATATTGCATTT-TGTCTGTTGT--T 1719
Cow2c4          ATGTATA--TTTCCTCCTTCGCACAGATGTATTACATATTGCATTT-TGTCTGTTGT--T 1719
Cow133c1        ATGTATA--TTTCCTCCTTCGCACAGATGTATTACATATTGCATTT-TGTCTGTTGT--T 1719
Cow139c9        ATGTATA--TTTCCTCCTTCGCACAGATGTATTACATATTGCATTT-TGTCTGTTGT--T 1719
Cow3535c3       ATGTATA--TTTCCTCCTTCGCACAGATGTATTACATATTGCATTT-TGTCTGTTGT--T 1719
Cow3535c6       ATGTATA--TTTCCTCCTTCGCACAGATGTATTACATATTGCATTT-TGTCTGTTGT--T 1719
Cow2c7          ATGTATA--TTTCCTCCTTCGCACAGATGTATTACATATTGCATTT-TGTCTGTTGT--T 1719
Cow2c10         ATGTATA--TTTCCTCCTTCGCACAGATGTATTACATATTGCATTT-TGTCTGTTGT--T 1719
AB007814Tth     ATGTATA--TTTCCTCCTTCGCACAGATGTATTACATATTGCATTT-TGTCTGTTGT--T 1719
Cow104c2        ATGTATA--TTTCCTCCTTCGCACAGATGTATTACATATTGCATTT-TGTCTGTTGT--T 1720
Cow104c3        ATGTATA--TTTCCTCCTTCGCACAGATGTATTACATATTGCATTT-TGTCTGTTGT--T 1720
Cow104c1        ATGTATA--TTTCCTCCTTCGCACAGATGTATTACATATTGCATTT-TGTCTGTTGT--T 1720
Cow2095c4       ATGTATG--TTTCCTCCTTCGCACAGATGTATTACATATTGCATTT-TGTCTGTTGT--T 1720
Cow2095c9       ATGTATA--TTTCCTCCTTCGCACAGATGTATTACATATTGCATTT-TGTCTGTTGT--T 1720
Cow2095c8       ATGTATA--TTTCCTCCTTCGCACAGATGTATTACATATTGCATTT-TGTCTGTTGT--T 1720
WTDA3c9.1       ATGTCAA--TTTCTTCCTTCGCACAGAT--ATTATAT-CTGTGTTT---TCTTTTGCGCC 1702
WTDA3c9.4       ATGTCAA--TTTCTTCCTTCGCACAGAT--ATTATAT-CTGTGTTT---TCTTTTGCGCC 1702
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Elk142c2        GTGTGTGGGTGT-----ATAACTCATACACG-GCCTCAGACAG-GTGCAATAACAAAAAA 1773
Elk142c10       GTGTGTGGGTGT-----ATAACTCATACACG-GCCTCAGACAG-GTGCAATAACAAAAAA 1776
Elk142c5        GTGTGTGGGTGT-----ATAACTCATACACG-GCCTCAGACAG-GTGCAATAACAAAAAA 1752
Elk328c3        GTGTGTGGGTGT-----ATAACCCATACACA-GCCGCAGACAGAGTGCAATAGCAAAAAA 1777
Elk416c8        GTGTGTGGGTGT-----ATAACCCATACACA-GCCGCAGACAGAGTGCAATAGCAAAAAA 1777
WTDN15c1        GTGTGTGGGTGTT----ATAACTCATACACA-GCTGCAGACAGAGTGCAATAGCAAAAAA 1781
WTDNL15c6       GTGTGTGGGTGTT----ATAACTCATACACA-GCTGCAGACAGAGTGCAATAGCAAAAAA 1777
WTDN15c9        GTGTGTGGGTGTT----ATAACTCATACACA-GCTGCAGACAGAGTGCAATAGCAAAAAA 1776
WTDA21c4        GTGTGTGGGTGTT----ATAACTCATACACA-GCCGCAGACAGAGTGCAATAGCAAAAAA 1781
WTDA21c5        GTGTGTGGGTGTT----ATAACTCATACACA-GCCGCAGACAGAGTGCAATAGCAAAAAA 1780
WTDA21c6        GTGTGTGGGTGT-----ATAACTCATGCACA-GCCGCAGACAGAGTGCAATAGCAAAAAA 1775
Cow139c10       GTGTGTGGGTGTAT---ATCTCTCATGCACA-GCCTCAGACAG--TGCAATAACAAAAAA 1773
Cow139c11       GTGTGTGGGTGTAT---ATCTCTCATGCACA-GCCTCAGACAG--TGCAATAACAAAAAA 1773
Cow2c4          GTGTGTGGGTGTAT---ATCTCTCACGCACA-GCCTCAGACAG--TGCAATAACAAAAAA 1773
Cow133c1        GTGTGTGGGTGTAT---ATCTCTCATGCACA-GCCTCAGACAG--TGCAATAACAAAAAA 1773
Cow139c9        GTGTGTGGGTGTAT---ATCTCTCATGCACA-GCCTCAGACAG--TGCAATAACAAAAAA 1773
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Cow3535c3       GTGTGTGGGTGTAT---ATCTCTCATGCACA-GCCTCAGACAG--TGCAATAACAAAAAA 1773
Cow3535c6       GCGTGTGGGTGTAT---ATCTCTCATGCACA-GCCTCAGACAG--TGCAATAACAAAAAA 1773
Cow2c7          GTGTGTGGGTGTAT---ATCTCTCATGCACA-GCCTCAGACAG--TGCAATAACAAAAAA 1773
Cow2c10         GTGTGTGGGTGTAT---ATCTCTCATGCACA-GCCTCAGACAG--TGCAATAACAAAAAA 1773
AB007814Tth     GTGTGTGGGTGTAT---ATCTCTCATGCACA-GCCTCAGACAG--TGCAATAACAAAAAA 1773
Cow104c2        GTGTGTGGGTGTAT---ATCTCTCATGCACA-GCCTCAGACAG--TGCAATAACAAAAAA 1774
Cow104c3        GTGTGTGGGTGTAT---ATCTCTCATGCACA-GCCTCAGACAG--TGCAATAACAAAAAA 1774
Cow104c1        GTGTGTGGGTGTAT---ATCTCTCATGCACA-GCCTCAGACAG--TGCAATAACAAAAAA 1774
Cow2095c4       GTGTGTGGGTGTATTATATCTCTCATGCACA-GCCTCAGACAG--TGCAATAACAAAAAA 1777
Cow2095c9       GTGTGTGGGTGTATTATATCTCTCATGCACA-GCCTCAGACAG--TGCAATAACAAAAAA 1777
Cow2095c8       GTGTGTGGGTGTATTATATCTCTCATGCACA-GCCTCAGACAG--TGCAATAACAAAAAA 1777
WTDA3c9.1       ATACCTAGTAGTC----ATA-CTTAGGTGTGTGATGCAAA-GGAAAACACAAACAAAAAAA 1756
WTDA3c9.4       ATACCTAGTAGTC----ATA-CTTAGGTGTGTGATGCAAA-GGAAAACACAAACAAAAAAA 1756
                     * *  **     **  *  *       *   ** *  *    **  * *******


Elk142c2        AA-CTCATGCCGCTTGACTTTCTCCACATAATACTTTATT---ATGTG-TGATTGTTGAG 1828
Elk142c10       A--CTCATGCCGCTTGACTTTCTCCACATAATACTTTATT---ATGTG-TGATTGTTGAG 1830
Elk142c5        AA-CTCATGCCGCTTGACTCTCTCCACATAATACTTTATT---ATGTG-TGATTGTTGAG 1807
Elk328c3        AA-CTCATGCCGCTTGACTCTC--CACATAAT----TAT------GTG-TGATTGTTGAG 1823
Elk416c8        AA-CTCATGCCGCTTGACTCTC--CACATAAT----TAT------GTG-TGATTGTTGAG 1823
WTDN15c1        AA-CTCATGCCGCTTGACTCTC--CACATAAT----TAT------GTG-TGATTGTTGAG 1827
WTDNL15c6       AA-CTCATGCCGCTTGACTCTC--CACATAAT----TAT------GTG-TGATTGTTGAG 1823
WTDN15c9        AA-CTCATGCCGCTTGACTCTC--CACATAAT----TAT------GTG-TGATTGTTGAG 1822
WTDA21c4        AA-CTCATGCCGCTTGACTCTC--CACATAAT----TAT------GTG-TGATTGTTGAG 1827
WTDA21c5        AA-CTCATGCCGCTTGACTCTC--CACATAAT----TAT------GTG-TGATTGTTGAG 1826
WTDA21c6        AAACTCATGCCGCTTGACTCTCTCCACATAAT----TATTGTTATGTG-TGATCGTTGAG 1830
Cow139c10       AA-CTCATGCCGCTTGACTCTCTTCACATAATAATTAATTATTATATGGTGATTGTTGAG 1832
Cow139c11       AAACTCATGCCGCTTGACTCTCTTCACATAATAATTAATTATTATATGGTGATTGTTGAG 1833
Cow2c4          AAACTCATGCCGCTTGACTCTCTTCACATAATAATTAATTATTATATGGTGATTGTTGAG 1833
Cow133c1        AA-CTCATGCCGCTTGACTCTCTTCACATAATAATTAATTATTATATGGTGATTGTTGAG 1832
Cow139c9        AA-CTCATGCCGCTTGACTCTCTTCACATAATAATTAATTATTATATGGTGATTGTTGAG 1832
Cow3535c3       AA-CTCATGCCGCTTGACTCTCTTCACATAATAATTAATTATTATATGGTGATTGTTGAG 1832
Cow3535c6       AA-CTCATGCCGCTTGACTCTCTTCACATAATAATTAATTATTATATGGTGATTGTTGAG 1832
Cow2c7          AA-CTCATGCCGCTTGACTCTCTTCACATAATAATTAATTATTATATGGTGATTGTTGAG 1832
Cow2c10         AA-CTCATGCCGCTTGACTCTCTTCACATAATAATTAATTATTATATGGTGATTGTTGAG 1832
AB007814Tth     AA-CTCATGCCGCTTGACTCTCTTCACATAATAATTAATTATTATATGGTGATTGTTGAG 1832
Cow104c2        AA-CTCATGCCGCTTGACTTTCTTCACATAATAATTAATTATTATGTGGTGATTGTTGAG 1833
Cow104c3        AA-CTCATGCCGCTTGACTTTCTTCACATAATAATTAATTATTATGTGGTGATTGTTGAG 1833
Cow104c1        AA-CTCATGCCGCTTGACTTTCTTCACATAATAATTAATTATTATGTGGTGATTGTTGAG 1833
Cow2095c4       AA-CTCATGCCGCTTGACTTTCTTCACATAATAATTAATTATTATGTGGTGATTGTTGAG 1836
Cow2095c9       AA-CTCATGCCGCTTGACTCTCTTCACATAATAATTAATTATTATGTGGTGATTGTTGAG 1836
Cow2095c8       AA-CTCATGCCGCTTGACTCTCTTCACATAATAATTAATTATTATGTGGTGATTGTTGAG 1836
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WTDA3c9.1          AA-CTCATGCCGCTTGACTCTCTCCACATATTATTTTAT----GTGTG-TGATTGTTGAG 1810
WTDA3c9.4          AA-CTCATGCCGCTTGACTCTCTCCACATATTATTTTAT----GTGTG-TGATTGTTGAG 1810
                   *  **************** **  ****** *     **        ** **** ******


                                5.8S→
Elk142c2           AACGGCCCCAACAACGTGTCGCGATGGATGACTTGGCTTCCTATTTCGTTGAAGAACGCA 1888
Elk142c10          AACGGCCCCAACAACGCGTCGCGATGGATGACTTGGCTTCCTATTTCGTTGAAGAACGCA 1890
Elk142c5           AACGGCCCCAACAACGTGTCGCGATGGATGACTTGGCTTCCTATTTCGTTGAAGAACGCA 1867
Elk328c3           AACGGCCCCAACAACGTGTCGCGATGGATGACTTGGCTTCCTATTTCGTTGAAGAACGCA 1883
Elk416c8           AACGGCCCCAACAACGTGTCGCGATGGATGACTTGGCTTCCTATTTCGTTGAAGAACGCA 1883
WTDN15c1           AACGGCCCCAACA-CGTGTCGCGATGGATGACTTGGCTTCCTATTTCGTTGAAGAACGCA 1886
WTDNL15c6          AACGGCCCCAACA-CGTGTCGCGATGGATGACTTGGCTTCCTATTTCGTTGAAGAACGCA 1882
WTDN15c9           AACGGCCCCAACA-CGTGTCGCGATGGATGACTTGGCTTCCTATTTCGTTGAAGAACGCA 1881
WTDA21c4           AACGGCCCCAACAACGTGTCGCGATGGATGACTTGGCTTCCTATTTCGTTGAAGAACGCA 1887
WTDA21c5           AACGGCCCCAACAACGTGTCGCGATGGATGACTTGGCTTCCTATTTCGTTGAAGAACGCA 1886
WTDA21c6           AACGGCCCCAACAACGTGTCGCGATGGATGACTTGGCTTCCTATTTCGTTGAAGAACGCA 1890
Cow139c10          AACGGCCCCAACAACGTGTCGCGATGGATGACTTGGCTTCCTATTTCGTTGAAGAACGCA 1892
Cow139c11          AACGGCCCCAACAACGTGTCGCGATGGATGACTTGGCTTCCTATTTCGTTGAAGAACGCA 1893
Cow2c4             AACGGCCCCAACAACGTGTCGCGATGGATGACTTGGCTTCCTATTTCGTTGAAGAACGCA 1893
Cow133c1           AACGGCCCCAACAACGTGTCGCGATGGATGACTTGGCTTCCTATTTCGTTGAAGAACGCA 1892
Cow139c9           AACGGCCCCAACAACGTGTCGCGATGGATGACTTGGCTTCCTATTTCGTTGAAGAACGCA 1892
Cow3535c3          AACGGCCCCAACAACGTGTCGCGATGGATGACTTGGCTTCCTATTTCGTTGAAGAACGCA 1892
Cow3535c6          AACGGCCCCAACAACGTGTCGCGATGGATGACTTGGCTTCCTATTTCGTTGAAGAACGCA 1892
Cow2c7             AACGGCCCCAACAACGTGTCGCGATGGATGACTTGGCTTCCTATTTCGTTGAAGAACGCA 1892
Cow2c10            AACGGCCCCAACAACGTGTCGCGATGGATGACTTGGCTTCCTATTTCGTTGAAGAACGCA 1892
AB007814Tth        AACGGCCCCAACAACGTGTCGCGATGGATGACTTGGCTTCCTATTTCGTTGAAGAACGCA 1892
Cow104c2           AACGGCCCCAACAACGTGTCGCGATGAATGACTTGGCTTCCTATTTCGTTGAAGAACGCA 1893
Cow104c3           AACGGCCCCAACAACGTGTCGCGATGAATGACTTGGCTTCCTATTTCGTTGAAGAACGCA 1893
Cow104c1           AACGGCCCCAACAACGTGTCGCGATGGATGACTTGGCTTCCTATTTCGTTGAAGAACGCA 1893
Cow2095c4          AACGGCCCCAACAACGTGTCGCGATGGATGACTTGGCTTCCTATTTCGTTGAAGAACGCA 1896
Cow2095c9          AACGGCCCCAACAACGTGTCGCGATGGATGACTTGGCTTCCTATTTCGTTGAAGAACGCA 1896
Cow2095c8          AACGGCCCCAACAACGTGTCGCGATGGATGACTTGGCTTCCTATTTCGTTGAAGAACGCA 1896
WTDA3c9.1          AACGGCCCCAACAACGTGTCGCGATGGATGACTTGGCTTCCTATTTCGTTGAAGAACGCA 1870
WTDA3c9.4          AACGGCCCCAACAACGTGTCGCGATGGATGACTTGGCTTCCTATTTCGTTGAAGAACGCA 1870
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Elk142c2           GCAAAGTGCGATAAGTGGTATCAATTGCAGAATCATTCAATTACCGAATCTTTGAACGCA 1948
Elk142c10          GCAAAGTGCGATAAGTGGTATCAATTGCAGAATCATTCAATTACCGAATCTTTGAACGCA 1950
Elk142c5           GCAAAGTGCGATAAGTGGTATCAATTGCAGAATCATTCAATTACCGAATCTTTGAACGCA 1927
Elk328c3           GCAAAGTGCGATAAGTGGTATCAATTGCAGAATCATTCAATTACCGAATCTTTGAACGCA 1943
Elk416c8           GCAAAGTGCGATAAGTGGTGTCAACTGCAGAATCATTCAATTACCGAATCTTTGAACGCA 1943
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WTDN15c1        GCAAAGTGCGATAAGTGGTATCAATTGCAGAATCATTCAATTACCGAATCTTTGAACGCA 1946
WTDNL15c6       ACAAAGTGCGATAAGTGGTATCAATTGCAGAATCATTCAATTACCGAATCTTTGAACGCA 1942
WTDN15c9        GCAAAGTGCGATAAGTGGTATCAATTGCAGAATCATTCAATTACCGAATCTTTGGACGCA 1941
WTDA21c4        GCAAAGTGCGATAAGTGGTATCAATTGCAGAATCATTCAATTACCGAATCTTTGAACGCA 1947
WTDA21c5        GCAAAGTGCGATAAGTGGTATCAATTGCAGAATCATTCAATTACCGAATCTTTGAACGCA 1946
WTDA21c6        GCAAAGTGCGATAAGTGGTACCAATTGCAGAATCATTCAATTACCGAATCTTTGAACGCA 1950
Cow139c10       GCAAAGTGCGATAAGTGGTATCAATTGCAGAATCATTCAATTACCGAATCTTTGAACGCA 1952
Cow139c11       GCAAAGTGCGATAAGTGGTATCAATTGCAGAATCATTCAATTACCGAATCTTTGAACGCA 1953
Cow2c4          GCAAAGTGCGATAAGTGGTATCAATTGCAGAATCATTCAATTACCGAATCTTTGAACGCA 1953
Cow133c1        GCAAAGTGCGATAAGTGGTATCAATTGCAGAATCATTCAATTACCGAATCTTTGAACGCA 1952
Cow139c9        GCAAAGTGCGATAAGTGGTATCAATTGCAGAATCATTCAATTACCGAATCTTTGAACGCA 1952
Cow3535c3       GCAAAGTGCGATAAGTGGTATCAATTGCAGAATCATTCAATTACCGAATCTTTGAACGCA 1952
Cow3535c6       GCAAAGTGCGATAAGTGGTATCAATTGCAGAATCATTCAATTACCGAATCTTTGAACGCA 1952
Cow2c7          GCAAAGTGCGATAAGTGGTATCAATTGCAGAATCATTCAATTACCGAATCTTTGAACGCA 1952
Cow2c10         GCAAAGTGCGATAAGTGGTATCAATTGCAGAATCATTCAATTACCGAATCTTTGAACGCA 1952
AB007814Tth     GCAAAGTGCGATAAGTGGTATCAATTGCAGAATCATTCAATTACCGAATCTTTGAACGCA 1952
Cow104c2        GCAAAGTGCGATAAGTGGTATCAATTGCAGAATCATTCAATTACCGAATCTTTGAACGCA 1953
Cow104c3        GCAAAGTGCGATAAGTGGTATCAATTGCAGAATCATTCAATTACCGAATCTTTGAACGCA 1953
Cow104c1        GCAAAGTGCGATAAGTGGTRTCAATTGCAGAATCATTCAAYTACCGAATCTTTGAACGCA 1953
Cow2095c4       GCAAAGTGCGATAAGTGGTATCAATTGCAGAATCATTCAATTACCGAATCTTTGAACGCA 1956
Cow2095c9       GCAAAGTGCGATAAGTGGTATCAATTGCAGAATCATTCAATTACCGAATCTTTGAACGCA 1956
Cow2095c8       GCAAAGTGCGATAAGTGGTATCAATTGCAGAATCATTCAATTACCGAATCTTTGAACGCA 1956
WTDA3c9.1       GCAAAGTGCGATAAGTGGTATCAATTGCAGAATCATTCAATTACCGAATCTTTGAACGCA 1930
WTDA3c9.4       GCAAAGTGCGATAAGTGGTATCAATTGCAGAATCATTCAATTACCGAATCTTTGAACGCA 1930
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Elk142c2        AACGGCGCATGGGAGAAGCTCCTCGGAGTCATCCCCGTGCATGCCATATTTCTCAGTGTC 2008
Elk142c10       AACGGCGCATGGGAGAAGCTCCTCGGAGTCATCCCCGTGCATGCCATATTTCTCAGTGTC 2010
Elk142c5        AACGGCGCATGGGAGAAGCTCCTCGGAGTCATCCCCGTGCATGCCATATTTCTCAGTGTC 1987
Elk328c3        AACGGCGCATGGGAGAAGCTCCTCGGAGTCATCCCCGTGCATGCCATATTTCTCAGTGTC 2003
Elk416c8        AACGGCGCATGGGAGAAGCTCCTCGGAGTCATCCCCGTGCATGCCATATTTCTCAGTGTC 2003
WTDN15c1        AACGGCGCATGGGAGAAGCTCCTCGGAGTCATCCCCGTGCATGCCATATTTCTCAGTGTC 2006
WTDNL15c6       AACGGCGCATGGGAGAAGCTCCTCGGAGTCATCCCCGTGCATGCCATATTTCTCAGTGTC 2002
WTDN15c9        AACGGCGCATGGGAGAAGCTCCTCGGAGTCATCCCCGTGCATGCCATATTTCTCAGTGTC 2001
WTDA21c4        AACGGCGCATGGGAGAAGCTCCTCGGAGTCATCCCCGTGCATGCCATATTTCTCAGTGTC 2007
WTDA21c5        AACGGCGCATGGGAGAAGCTCCTCGGAGTCATCCCCGTGCATGCCATATTTCTCAGTGTC 2006
WTDA21c6        AACGGCGCATGGGAGAAGCTCCTCGGAGTCATCCCCGTGCATGCCATATTTCTCAGTGTC 2010
Cow139c10       AACGGCGCACGGGAGAAGCTCCTCGGAGTCATCCCCGTGCATGCCATATTTCTCAGTGCC 2012
Cow139c11       AACGGCGCATGGGAGAAGCTCCTCGGAGTCATCCCCGTGCATGCCATATTTCTCAGTGTC 2013
Cow2c4          AACGGCGCATGGGAGAAGCTCCTCGGAGTCATCCCCGTGCATGCCATATTTCTCAGTGTC 2013
Cow133c1        AACGGCGCATGGGAGAAGCTCCTCGGAGTCATCCCCGTGCATGCCATATTTCTCAGTGTC 2012
Cow139c9        AACGGCGCATGGGAGAAGCTCCTCGGAGTCATCCCCGTGCATGCCATATTTCTCAGTGTC 2012
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Cow3535c3      AACGGCGCATGGGAGAAGCTCCTCGGAGTCATCCCCGTGCATGCCATATTTCTCAGTGTC 2012
Cow3535c6      AACGGCGCATGGGAGAAGCTCCTCGGAGTCATCCCCGTGCATGCCATATTTCTCAGTGTC 2012
Cow2c7         AACGGCGCATGGGAGAAGCTCCTCGGAGTCATCCCCGTGCATGCCATATTTCTCAGTGTC 2012
Cow2c10        AACGGCGCATGGGAGAAGCTCCTCGGAGTCATCCCCGTGCATGCCATATTTCTCAGTGTC 2012
AB007814Tth    AACGGCGCATGGGAGAAGCTCCTCGGAGTCATCCCCGTGCATGCCATATTTCTCAGTGTC 2012
Cow104c2       AACGGCGCATGGGAGAAGCTCCTCGGAGTCATCCCCGTGCATGCCATATTTCTCAGTGTC 2013
Cow104c3       AACGGCGCATGGGAGAAGCTCCTCGGAGTCATCCCCGTGCATGCCATATTTCTCAGTGTC 2013
Cow104c1       AACGGCGCATGGGAGAAGCTCCTCGGAGTCATCCCCGTGCATGCCATATTTCTCAGTGTC 2013
Cow2095c4      AACGGCGCATGGGAGAAGCTCCTCGGAGTCATCCCCGTGCATGCCATATTTCTCAGTGTC 2016
Cow2095c9      AACGGCGCATGGGAGAAGCTCCTCGGAGTCATCCCCGTGCATGCCATATTTCTCAGTGTC 2016
Cow2095c8      AACGGCGCATGGGAGAAGCTCCTCGGAGTCATCCCCGTGCATGCCATATTTCTCAGTGTC 2016
WTDA3c9.1      AACGGCGCATGGGAGAAGCTCCCCGGAGTCATCCCCGTGCATGCCATATTTCTCAGTGTC 1990
WTDA3c9.4      AACGGCGCATGGGAGAAGCTCCCCGGAGTCATCCCCGTGCATGCCATATTTCTCAGTGTC 1990
               ********* ************ *********************************** *


                 ITS2→
Elk142c2       GAACAACAAAAAACCACCATACAAACATGTTATTATGTATGTGTGTGTGGGAATTTTAAA 2068
Elk142c10      GAACAACAAAAAAACCACCATACAAACATGTTATTGTGTATGTGTGTGTGGGAATTTTAAA 2070
Elk142c5       GAACAACAAAAAAACCACCATACAAACATGTTATTGTGTATGTGTGTGTGGGAATTTTAAA 2047
Elk328c3       GAACAACAAAAAAACCACCATACAAACATGTTATTGTGTATGTGTGTGTGGGAATTTTAAA 2063
Elk416c8       GAACAACAAAAAAACCACCATACAAACATGTTATTGTGTATGTGTGTGTGGGAATTTTAAA 2063
WTDN15c1       GAA-AACAAAAAAACCACCATACAAACATGTTATTGTGTATGTGTGTGTGGGAATTTTAAA 2065
WTDNL15c6      GAA-AGCAAAAAAACCACCATATAAACATGTTATTGTGTATGTGTGTGTGGGAATTTTAAA 2061
WTDN15c9       GAA-AACAAAAAAACCACCATACAAACATGTTATTGTGTATGTGTGTGTGGGAATTTTAAA 2060
WTDA21c4       GAACAACAAAAAAACCACCATACAAACATGTTATTGTGTATGTGTGTGTGGGAATTTTAAA 2067
WTDA21c5       GAACAACAAAAAAACCACCATACAAACATGTTATTGTGTATGTGTGTGTGGGAATTTTAAA 2066
WTDA21c6       GAACAACAAAAAAACCACCATACAAACATGTTATTGTGTATGTGTGTGTGGGAATTTTAAA 2070
Cow139c10      GAACAACAAAAAAACCACCATACAAACATGTTATTGTGTATGTGTGTGTGGGAATTTTAAA 2072
Cow139c11      GAACAACAAAAAAACCACCATACAAACATGTTATTGTGTATGTGTGTGTGGGAATTTTAAA 2073
Cow2c4         GAACAACAAAAAAACCACCATACAAACATGTTATTGTGTATGTGTGTGTGGGAATTTTAAA 2073
Cow133c1       GAACAACAAAAAAACCACCATACAAACATGTTATTGTGTATGTGCGTGTGGGAATTTTAAA 2072
Cow139c9       GAACAACAAAAAAACCACCATACAAACATGTTATTGTGTATGTGTGTGTGGGAATTTTAAA 2072
Cow3535c3      GAACAACAAAAAAACCACCATACAAACATGTTATTGTGTATGTGTGTGTGGGAATTTTAAA 2072
Cow3535c6      GAACAACAAAAAAACCACCATACAAACATGTTATTGTGTATGTGTGTGTGGGAATTTTAAA 2072
Cow2c7         GAACAACAAAAAAACCACCATACAAACATGTTATTGTGTATGTGTGTGTGGGGATTTTAAA 2072
Cow2c10        GAACAACAAAAAAACCACCATACAAACATGTTATTGTGTATGTGTGTGTGGGGATTTTAAA 2072
AB007814Tth    GAACAACAAAAAAACCACCATACAAACATGTTATTGTGTATGTGTGTGTGGGAATTTTAAA 2072
Cow104c2       GAACAACAAAAAAACCACCATACAAACATGTTATTGTGTATGTGTGTGTGGGAATTTTAAA 2073
Cow104c3       GAACAACAAAAAAACCACCATACAAACATGTTATTGTGTATGTGTGTGTGGGAATTTTAAA 2073
Cow104c1       GAACAACAAAAAAACCACCATACAAACATGTTATTGTGTATGTGTGTGTGGGAATTTTAAA 2073
Cow2095c4      GAACAACAAAAAAACCACCATACAAACATGTTATTGTGTATGTGTGTGTGGGAATTTTAAA 2076
Cow2095c9      GAACAACAAAAAAACCACCATACAAACATGTTATTGTGTATGTGTGTGTGGGAATTTTAAA 2076
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Cow2095c8        GAACAACAAAAAACCACCATACAAACACGTTATTGTGTATGTGTGTGTGGGAATTTTAAA 2076
WTDA3c9.1        GAACAACAAAAAACCACCATACAAACATGTTATTGTGTATGTGTGTGTGGGAATTTTAAA 2050
WTDA3c9.4        GAACAACAAAAAACCACCATACAAACATGTTATTGTGTATGTGTGTGTGGGAATTTTAAA 2050
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Elk142c2         CAGAGAGCCCTTGGGGATACGGATGAAACACATTCTTCAGCGTGCGTTTACTCGCATGCA 2128
Elk142c10        CAGAGAGCCCTTGGGGATACGGATGAAACACACTCTTCAGCGTGCGTTTACTCGCATGCA 2130
Elk142c5         CAGAGAGCCCTTGGGGATACGGATGAAACACACTCTTCAGCGTGCGTTTACTCGCATGCA 2107
Elk328c3         CAGAGAGCCCTTGGGGATACGGATGAAACACACTCTTCAGCGCGCGTTTACTCGCATGCA 2123
Elk416c8         CAGAGAGCCCTTGGGGATACGGATGAAACACACTCTTCAGCGCGCGTTTACTCGCATGCA 2123
WTDN15c1         CAGAGAGCCCTTGGGGATACGGATGAAACACACTCTTCAGCGCGCGTTTACTCGCATGCA 2125
WTDNL15c6        CAGAGAGCCCTTGGGGATACGGATGAAACACACTCTTCAGCGCGCGTTTACTCGCATGCA 2121
WTDN15c9         CAGAGAGCCCTTGGGGATACGGATGAAACACACTCTTCAGCGCGCGTTTACTCGCATGCA 2120
WTDA21c4         CAGAGAGCCCTTGGGGATACGGATGAAACACACTCTTCAGCGCGCGTTTACTCGCATGCA 2127
WTDA21c5         CAGAGAGCCCTTGGGGATACGGATGAAACACACTCTTCAGCGCGCGTTTACTCGCATGCA 2126
WTDA21c6         CAGAGAGCCCTTGGGGATACGGATGAAACACACTCTTCAGCGCGCGTTTACTCGCATGCA 2130
Cow139c10        CAGAGAGCCCTTGGGGATACGGGTGAAACACACTCTCCGGCACGCGTTTACTCGCATGCA 2132
Cow139c11        CAGAGAGCCCTTGGGGATACGGATGAAACACACTCTCCGGCACGCGTTTACTCGCATGCA 2133
Cow2c4           CAGAGAGCCCTTGGGGATACGGATGAAACACACTCTCCGGCACGCGTTTACTCGCATGCA 2133
Cow133c1         CAGAGAGCCCTTGGGGATACGGATGAAACACACTCTCCGGCACGCGTTTACTCGCATGCA 2132
Cow139c9         CAGAGAGCCCTTGGGGATACGGATGAAACACACTCTCCGGCACGCGTTTACTCGCATGCA 2132
Cow3535c3        CAGAGAGCCCTTGGGGATACGGATGAAACACACTCTCCGGCACGCGTTTACTCGCATGCA 2132
Cow3535c6        CAGAGAGCCCTTGGGGATACGGATGAAACACACTCTCCGGCACGCGTTTACTCGCATGCA 2132
Cow2c7           CAGAGAGCCCTTGGGGATACGGATGAAACACACTCTCCGGCACGCGTTTACTCGCATGCA 2132
Cow2c10          CAGAGAGCCCTTGGGGATACGGATGAAACACACTCTCCGGCACGCGTTTACTCGCATGCA 2132
AB007814Tth      CAGAGAGCCCTTGGGGATACGGATGAAACACACTCTCCGGCACGCGTTTACTCGCATGCA 2132
Cow104c2         CAGAGAGCCCTTGGGGATACGGATGAAACACACTCTCCGGCACGCGTTTACTCGCATGCA 2133
Cow104c3         CAGAGAGCCCTTGGGGATACGGATGAAACACACTCTCCGGCACGCGTTTACTCGCATGCA 2133
Cow104c1         CAGAGAGCCCTTGGGGATACGGATGAAACACACTCTCCGGCACGCGTTTACTCGCATGCA 2133
Cow2095c4        CAGAGAGCCCTTGGGGATACGGATGAAACACACTCTCCGGCACGCGTTTACTCGCATGCA 2136
Cow2095c9        CAGAGAGCCCTTGGGGATACGGATGAAACACACTCTCCGGCACGCGTTTACTCGCATGCA 2136
Cow2095c8        CAGAGAGCCCTTGGGGATACGGATGAAACACACTCTCCGGCACGCGTTTACTCGCATGCA 2136
WTDA3c9.1        CAGAGAGCCCTTGGGGATACGGATGAAACATA---------------------------- 2082
WTDA3c9.4        CAGAGAGCCCTTGGGGATACGGATGAAACATA---------------------------- 2082
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 Elk142c2        GAA-GAGGGAACTGCACTATTTTTT-AGTGTGGTTTTTTTGTGTATGATCCGCTCCGGCG 2186
Elk142c10        GAA-GAGGGAGCTGCACTATTTTTTTAGTGTGGTTTTTTTGTGTATGATCCGCTCCGGCG 2189
Elk142c5         GAA-GAGGGAGCTGCACTATTTTTTTAGTGTGGTTTTTTTGTGTATGATCCGCTCCGGCG 2166
Elk328c3         GAA-GAGGGAACTATACTATTCTTT-AGTGTGGTTTTTTTGTGTATGATCCGCTCCGGCG 2181
Elk416c8         GAA-GAGGGAACTATACTATTCTTT-AGTGTGGTTTTTTTGTGTATGATCCGCTCCGGCG 2181
WTDN15c1         GAA-GATGGAACTATACTATTTTTT-AGTGTGGTTTTTCGTGTATGATCCGCTCCGGCG 2183
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WTDNL15c6      GAA-GATGGAACTATACTATTTTTT-AGTGTGGTTTTTTCGTGTATGATCCGCTCCGGCG 2179
WTDN15c9       GAA-GATGGAACTATACTATTTTTT-AGTGTGGTTTTTTCGTGTATGATCCGCTCCGGCG 2178
WTDA21c4       GAA-GAGGGAACTATACTATTTTTT-AGTGTGGTTTTTTCGTGTATGATCCGCTCCGGCG 2185
WTDA21c5       GAA-GAGGGAACTATACTATTTTTT-AGTGTGGTTTTTTCGTGTATGATCCGCTCCGGCG 2184
WTDA21c6       GAA-GAGGGAACTATACTATTTTTT-AGTGTGGTTTTTTCGTGTATGATCCGCTCCGGCG 2188
Cow139c10      GAAAGAGAGGGCTACACTATTTTTTTAGTGTGGTATTTTTGTGTATGATCCGCTCCGGCG 2192
Cow139c11      GAAAGAGAGGGCTACACTATTTTTTTAGTGTGGTATTTTTGTGTATGATCCGCTCCGGCG 2193
Cow2c4         GAAAGAGAGGGCTACACTATTTTTTTAGTGTGGTATTTTTGTGTATGATCCGCTCCGGCG 2193
Cow133c1       GAAAGAGAGGGCTACACTATTTTTTTAGTGTGGTATTTTTGTGTATGATCCGCTCCGGCG 2192
Cow139c9       GAAAGAGAGGGCTACACTATTTTTTTAGTGTGGTATTTTTGTGTATGATCCGCTCCGGCG 2192
Cow3535c3      GAAAGAGAGGGCTACACTATTTTTTTAGTGTGGTATTTTTGTGTATGATCCGCTCCGGCG 2192
Cow3535c6      GAAAGAGAGGGCTACACTATTTTTTTAGTGTGGTATTTTTGTGTATGATCCGCTCCGGCG 2192
Cow2c7         GAAAGAGAGGGCTACACTATTTTTTTAGTGTGGTATTTTTGTGTATGATCCGCTCCGGCG 2192
Cow2c10        GAAAGAGAGGGCTACACTATTTTTTTAGTGTGGTATTTTTGTGTATGATCCGCTCCGGCG 2192
AB007814Tth    GAAAGAGAGGGCTACACTATTTTTTTAGTGTGGTATTTTTGTGTATGATCCGCTCCGGCG 2192
Cow104c2       GAAAGAGAGGGCTACACTATTTTTTTAGTGTGGTATTTTTGTGTATGATCCGCTCCGGCG 2193
Cow104c3       GAAAGAGAGGGCTACACTATTTTTTTAGTGTGGTATTTTTGTGTATGATCCGCTCCGGCG 2193
Cow104c1       GAAAGAGAGGGCTACACTATTTTTTTAGTGTGGTATTTTTGTGTATGATCCGCTCCGGCG 2193
Cow2095c4      GAAAGAGAGGGCTACACTATTTTTTTAGTGTGGTATTTTTGTGTATGATCCGCTCCGGCG 2196
Cow2095c9      GAAAGAGAGGGCTACACTATTTTTTTAGTGTGGTATTTTTGTGTATGATCCGCTCCGGCG 2196
Cow2095c8      GAAAGAGAGGGCTACACTATTTTTTTAGTGTGGTATTTTTGTGTATGATCCGCTCCGGCG 2196
WTDA3c9.1      ------------------------------------TTTTGTGTATGATCCGCTCCGGCG 2106
WTDA3c9.4      ------------------------------------TTTTGTGTATGATCCGCTCCGGCG 2106
                                                   *** ********************


Elk142c2       CTTATGTGTGTGTGCGTGTG--CTCCTTCCTCTCTCACTTTGTGTACTCATGT-----AT 2239
Elk142c10      CTTATGTGTGTGTGCGTGTG--CTCCTTCCTCTCTCACTTTGTGTACTCATGT-----AT 2242
Elk142c5       CTTATGTGTGTGTGCGTGTG--CTCCTTCCTCTCTCACTTTGTGTACTCATGT-----AT 2219
Elk328c3       CTTGTGTGTGTG--CGTGTG--CTCCTTCCTCTCTCACTTTGTGCTCATATGT-----GT 2232
Elk416c8       CTTGTGTGTGTGTGCGTGTG--CTCCTTCCTCTCTCACTTTGTGCTCATATGT-----GT 2234
WTDN15c1       CTTGTGTGTGTGTGCGTGTG--CTCCTTCCTCTCTCACTTTGTGCTCATATGT-----GT 2236
WTDNL15c6      CTTGTGTGTGTGTGCGTGTG--CTCCTTCCTCTCTCACTTTGTGCTCATATGT-----GT 2232
WTDN15c9       CTTGTGTGTGTGTGCGTGTG--CTCCTTCCTCTCTCACTTTGTGCTCATATGT-----GT 2231
WTDA21c4       CTTGTGTGTGTGTGCGTGTG--CTCCTTCCTCTCTCACTTTGTGCTCATATGT-----GT 2238
WTDA21c5       CTTGTGTGTGTGTGCGTGTG--CTCCTTCCTCTCTCACTTTGTGCTCATATAT-----GT 2237
WTDA21c6       CTTGTGTGTGTGTGCGTGTG--CTCCTTCCTCTCTCTCTTTGTGCTCATATAT-----GT 2241
Cow139c10      CTTGTGTGTGTGTGCGTGTA--CTCCTTCCTCTCTCACTTTGTACTCATATGT-----AT 2245
Cow139c11      CTTGTGTGTGTGTGCGTGTG--CTCCTTCCTCTCTCACTTTGTACTCATATGT-----AT 2246
Cow2c4         CTTGTGTGTGTGTGCGTGTG--CTCCTTCCTCTCTCACTTTGTACTCATATGT-----AT 2246
Cow133c1       CTTGTGTGTGTGTGCGTGTG--CTCCTTCCTCTCTCACTTTGTACTCATATGT-----AT 2245
Cow139c9       CTTGTGTGTGTGTGCGTGTG--CTCCTTCCTCTCTCACTTTGTACTCATATGT-----AT 2245
Cow3535c3      CTTGTGTGTGTGTGCGTGTG--CTCCTTCCTCTCTCACTTTGTACTCATATGT-----AT 2245
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Cow3535c6        CTTGTGTGTGTGCGCGTGTG--CTCCTTCCTCTCTCACTTTGTACTCATATGT-----AT 2245
Cow2c7           CTTGTGTGTGTGTGCGTGTG--CTCCTTCCTCTCTCACTTTGTACTCACATGT-----AT 2245
Cow2c10          CTTGTGTGTGTGTGCGTGTG--CTCCTTCCTCTCTCACTTTGTACTCATATGT-----AT 2245
AB007814Tth      CTTGTGTGTGTGTGCGTGTG--CTCCTTCCTCTCTCACTTTGTACTCATATGT-----AT 2245
Cow104c2         CTTGTGTGTGTGTGCGTGTG--CTCCTTCCTCTCTCACTTTGTACTCATATGT-----AT 2246
Cow104c3         CTTGTGTGTGTGTGCGTGTG--CTCCTTCCTCTCTCACTTTGTACTCATATGT-----AT 2246
Cow104c1         CTTGTGTGTGTGTGCGTGTG--CTCCTTCCTCTCTCACTTTGTACTCATATGT-----AT 2246
Cow2095c4        CTTGTGTGTGTGTGCGTGTG--CTCCTTCCTCTCTCACTTTATACTCATATGT-----AT 2249
Cow2095c9        CTTGTGTGTGTGTGCGTGTG--CTCCTTCCTCTCTCACTTTATACTCATATGT-----AT 2249
Cow2095c8        CTTGTGTGTGTGTGCGTGTG--CTCCTTCCTCTCTCACTTTGTACTCATATGT-----AT 2249
WTDA3c9.1        CTTGTGTGTGTGTGCGTGTGTTCTCTTTCCTCTCTCACATTTTGCTCGTGTGTGTAAAAT 2166
WTDA3c9.4        CTTGTGTGTGTGTGCGTGTGTTCTCTTTCCTCTCTCACATTTTGCTCGTGTGTGTAAAAT 2166
                 *** ********   *****   *** ********** * ** *   *   * *    *

Elk142c2         AT--------------------------GAATTGAGAAAAAGGGGGAATTATACCACATA 2273
Elk142c10        AT--------------------------GAATTGAGAAAAAGGGGGAATTATACCACATA 2276
Elk142c5         AT--------------------------GAATTGAGAAAAAGGGGGAGTTATACCACATA 2253
Elk328c3         AT--------------------------GAATTGAGAAAAAGGGGGAATCATACCACATA 2266
Elk416c8         AT--------------------------GAATTGAGAAAAAGGGGGAATCATACCACATA 2268
WTDN15c1         AT--------------------------GAATTGAGAAAAAGGGGGAACCATACCACATA 2270
WTDNL15c6        AT--------------------------GAATTGGGAAAAAGGGGGAATCATACCACATA 2266
WTDN15c9         AC--------------------------GAATTGGGAAAAAGGGGGAATCATACCACATA 2265
WTDA21c4         AT--------------------------GAATTGAGAAAAAGGGGGAATTATACCACATA 2272
WTDA21c5         AT--------------------------GAATTGAGAAAAAGGGGGAATTATACCACATA 2271
WTDA21c6         ATTTATTTATATATA----TGTGTGTATGAATTGAGAAAAAGGGGGAATCATACCACATA 2297
Cow139c10        ATGTGTGTGT------------------AAATTGAGAAAA-GGGGGAATCATACCACATA 2286
Cow139c11        ATGTGTGTGT------------------AAATTGAGAAAAAGGGGGAATCATACCACATA 2288
Cow2c4           ATGTGTGTGT------------------AAATTGAGAAAAAGGGGGAATCATACCACATA 2288
Cow133c1         ATGTGTGTGT------------------AAATTGAGAAAAAGGGGGAATCATACCACATA 2287
Cow139c9         ATGTGTGTGT------------------AAATTGAGAAAAAGGGGGAATCATACCACATA 2287
Cow3535c3        ATGTGTGTGT------------------AAATTGAGAAAAAGGGGGAATCATACCACATA 2287
Cow3535c6        ATGTGTGTGT------------------AAATTGAGAAAAAGGGGGAATCATACCACATA 2287
Cow2c7           ATGTGTGTGT------------------AAATTGAGAAAAAGGGGGAATCATACCACATA 2287
Cow2c10          ATGTGTGTGT------------------AAATTGAGAAAAAGGGGGAATCATACCACATA 2287
AB007814Tth      ATGTGTGTGT------------------AAATTGAGAAAAAGGGGGAATCATACCACATA 2287
Cow104c2         ATGTGTGTGT------------------AAATTGAGAAAAAGGGGGAATCATACCACATA 2288
Cow104c3         ATGTGTGTGT------------------AAATTGAGAAAAAGGGGGAATCATACCACATA 2288
Cow104c1         ATGTGTGTGT------------------AAATTGAGAAAAAGGGGGAATCATACCACATA 2288
Cow2095c4        ATGTGTGTGT------------------GAATTGAGAAAAAGGGGGAATCATACCACATA 2291
Cow2095c9        ATGTGTGTGT------------------AAATTGAGAAAAAGGGGGAATCATACCACATA 2291
Cow2095c8        ATGTGTGTGT------------------AAATTGAGAAAAAGGGGGAATCATACCACATA 2291
WTDA3c9.1        ATGTATATATTATATATATCATGAGTGTAAATTTGGGAAAAGGGGGGAACAAACCACATG 2226
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WTDA3c9.4          ATGTATATATTATATATATCATGAGTGTAAATTTGGGAAAAGGGGGGAACAAACCACATG 2226
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Elk142c2           CATGTCTGTACAACAACAACCAAAAACACCAACGAGAGAAAAAGATAA-AAAAGGATTG- 2331
Elk142c10          CATGTCTGTACAACAACAACCAAAAACACCAACGAGAGAAAAAGATAA-AAAAGGATTG- 2334
Elk142c5           CATGTCTGTACAACAACAACCAAAAACACCAACGAGAGAAAAAGATAA-AAAAGGATTG- 2311
Elk328c3           CATGTCTGTACAACAACAACCAAAAACACCAACGAGAGAAAAAGACAA-AAAAGGATTG- 2324
Elk416c8           CATGTCTGTACAACAACAACCAAAAACACCAACGAGAGAAAAAGACAA-AAAAGGATTG- 2326
WTDN15c1           CATGTCTGTACAACAACAACCAAAAACACCAACGAGAGAAAAAGATAA-AAAGGGATTG- 2328
WTDNL15c6          CATGTCTGTACAACAACAACCAAAAACACCAACGAGAGAAAAAGATAA-AAAAGGATTG- 2324
WTDN15c9           CATGTCTGTACAACAACAACCAAAAACACCAACGAGAGAAAAAGATAA-AAAAGGATTG- 2323
WTDA21c4           CATGTCTGTACAACAACAACCAAAAACACCAACGAGAGAAAAAGATAA-AAAAGGATTG- 2330
WTDA21c5           CATGTCTGTACAACAACAACCAAAAACACCAACGAGAGAGAAAGATAA-AAAAGGATTG- 2329
WTDA21c6           CATGTCTGTACAACAACAACCAAAAACACCAACGAGAGAAAAAGATAA-AAAAGGATTG- 2355
Cow139c10          CATGTCTGTACAACAACAACCAAAAACACCAACGAGAGAAAAAGATAAGAAAAGGATTG- 2345
Cow139c11          CATGTCTGTACAACAACAACCAAAAACACCAACGAGAGAAAAAGATAAGAAAAGGATTG- 2347
Cow2c4             CATGTCTGTACAACAACAACCAAAAACACCAACGAGAGAAAAAGATAAGAAAGGGATTG- 2347
Cow133c1           CATGTCTGTACAACAACAACCAAAAACACCAACGAGAGAAAAAGATAAGAAAAGGATTG- 2346
Cow139c9           CATGTCTGTACAACAACAACCAAAAACACCAACGAGAGAAAAAGATAAGAAAAGGATTG- 2346
Cow3535c3          CATGTCTGTACAACAACAACCAAAAACACCAACGAGAGAAAAAGATAAGAAAAGGATTG- 2346
Cow3535c6          CATGTCTGTACAACAACAACCAAAAACACCAACGAGAGAAAAAGATAAGAAAAGGATTG- 2346
Cow2c7             CATGTCTGTACAACAACAACCAAAAACACCAACGAGAGAAAAAGATAAGAAAAGGATTG- 2346
Cow2c10            CATGTCTGTACAACAACAACCAAAAACACCAACGAGAGAAAAAGATAAGAAAAGGATTG- 2346
AB007814Tth        CATGTCTGTACAACAACAACCAAAAACACCAACGAGAGAAAAAGATAAGAAAAGGATTG- 2346
Cow104c2           CATGTCTGTGCAACAACAACCAAAAACACCAACGAGAGAAAAAGATAAGAAAAGGATTG- 2347
Cow104c3           CATGTCTGTGCAACAACAACCAAAAACACCAACGAGAGAAAAAGATAAGAAAAGGATTG- 2347
Cow104c1           CATGTCTGTACAACAACAACCAAAAACACCAACGAGAGAAAAAGATAAGAAAAGGATTG- 2347
Cow2095c4          CATGTCTGTACAACAACAACCAAAAACACCAACGAGAGAAAAAGATAAGAAAAGGATTG- 2350
Cow2095c9          CATGTCTGTACAACAACAACCAAAAACACCAACGAGAGAAAAAGATAAGAAAAGGATTG- 2350
Cow2095c8          CATGTCTGTACAACAACAACCAAAAACACCAACGAGAGAAAAAGATAAGAAAAGGATTG- 2350
WTDA3c9.1          CATGTCTGTACAACAACAACCAAAAACACCAACGAGAGAAAAAGATAA--AAAGGATTGT 2284
WTDA3c9.4          CATGTCTGTACAACAACAACCAAAAACACCAACGAGAGAAAAAGATAA--AAAGGATTGT 2284
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Elk142c2           -GGGCTT--CGGTCTCTCTTCTT--TTTTTCTTTTCCTA--CGCCATGTGTGTATGGTAT 2384
Elk142c10          -GGGCTT--CGGTCTCTCTTCTT--TTTTTCTTTTCCTA--CGCCATGTGTGTATGGTAT 2387
Elk142c5           -TGGCTT--CGGTCTCTCTTCTT--TTTTTCTTTTCCTA--CGCCATGTGTGTATGGTAT 2364
Elk328c3           -GGGCTT--AGGTCTCTCTTCTT--TTTTTCTTTTCCTG--CGCCATGTGTGTATGGTAT 2377
Elk416c8           -GGGCTT--AGGTCTCTCTTCTT--TTTTTCTTTTCCTG--CGCCATGTGTGTATGGTAT 2379
WTDN15c1           -GGGCTT--AGGTCTCTCTTCTT--TTTTTCTTTTCCTG--CGCCATGTGTGTATGGTAT 2381
WTDNL15c6          -GGGCTT--AGGTCTCTCTTCTT--TTTTTCTTTTCCTG--CGCCATGTGTGTATGGTAT 2377
WTDN15c9           -GGGCTT--AGGTCTCTCTTCTT--TTTTTCCTTTCCTG--CGCCATGTGTGTATGGTAT 2376
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WTDA21c4        -GGGCTT--AGGTCTCTCTTCTT--TTTTTCTTTTCCTG--CGCCATGTGTGTATGGTAT 2383
WTDA21c5        -GGGCTT--AGGTCTCTCTTCTT--TTTTTCTTTTCCTG--CGCCATGTGTGTATGGTAT 2382
WTDA21c6        -GGACTT--AGGTCTCTCTTCTT--TTTTTCTTTTCCTG--CGCCATGTGTGTATGGTAT 2408
Cow139c10       -GGGCTT--AGGTCTCTCTTCTT-TTTTTTCTTTTCCTA--CGCCATGTGTGTATGGTAT 2399
Cow139c11       -GGGCTT--AGGTCTCTCTTCTT-TTTTTTCTTTTCCTA--CGCCATGTGTGTATGGTAT 2401
Cow2c4          -GGGCTT--AGGTCTCTCTTCTT-TTTTTTCTTTTCCTA--CGCCATGTGTGTATGGTAT 2401
Cow133c1        -GGGCTT--AGGTCTCTCTTCTT-TTTTTTCTTTTCCTA--CGCCATGTGTGTATGGTAT 2400
Cow139c9        -GGGCTT--AGGTCTCTCTTCTT-TTTTTTCTTTTCCTA--CGCCATGTGTGTATGGTAT 2400
Cow3535c3       -GGGCTT--AGGTCTCTCTTCTT-TTTTTTCTTTTCCTA--CGCCATGTGTGTATGGTAT 2400
Cow3535c6       -GGGCTT--AGGTCTCTCTTCTT-TTTTTTCTTTTCCTA--CGCCATGTGTGTATGGTAT 2400
Cow2c7          -GGGCTT--AGGTCTCTCTTCTT-TTTTT-CTTTTCCTA--CGCCATGTGTGTATGGTAT 2399
Cow2c10         -GGGCTT--AGGTCTCTCTTCTT-TTTTTTCTTTTCCTA--CGCCATGTGTGTATGGTAT 2400
AB007814Tth     -GGGCTT--AGGTCTCTCTTCTT-TTTTTTCTTTTCCTA--CGCCATGTGTGTATGGTAT 2400
Cow104c2        -GGGCTT--AGGTCTCTCTTCTTCTTTTTTCTTTTCCTA--CGCCATGTGTGTATGGTAT 2402
Cow104c3        -GGGCTT--AGGTCTCTCTTCTTCTTTTTTCTTTTCCTA--CGCCATGTGTGTATGGTAT 2402
Cow104c1        -GGGCTT--AGGTCTCTCTTCTTCTTTTTTCTTTTCCTA--CGCCATGTGTGTATGGTAT 2402
Cow2095c4       -GGGCTT--AGGTCTCTCTTCTT-TTTTTTCTTTTCCTA--CGCCATGTGTGTATGGTAT 2404
Cow2095c9       -GGGCTT--AGGTCTCTCTTCTT-TTTTTTCTTTTCCTA--CGCCATGTGTGTATGGTAT 2404
Cow2095c8       -GGGCTT--AGGTCTCTCTTCTT-TCTTTTCTTTTCCTA--CGCCATGTGTGTATGGTAT 2404
WTDA3c9.1       GGGGCTTTACGGTCTCTCTTTT-CTTTTTTCTTTTCCTGTGCGCCATGTGTGTATGGTAT 2343
WTDA3c9.4       GGGGCTTTACGGTCTCTCTTTT-CTTTTTTCTTTTCCTGTGCGCCATGTGTGTATGGTAT 2343
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Elk142c2        ATA--AAAATTATACTGT--GGGTGCGTGT---AACGCGTATCTGCAATCTTTGT-CA-- 2434
Elk142c10       ATA--AAAATTATACTGT--GGGTGCGTGT---AACGCGTATCTGCAATCTTTGT-CA-- 2437
Elk142c5        ATA--AAAATTATACTGT--GGGTGCGTGT---AACGCGTATCTGCAATCTTTGT-CA-- 2414
Elk328c3        ATA--AAAATTATACTGT--GGGTGCGTGT---TACGCGTATCTGCAATCTTTGT-CA-- 2427
Elk416c8        ATA--AAAACTATACTGT--GGGTGCGTGT---TACGCGTATCTGCAATCTTTGT-CA-- 2429
WTDN15c1        ATA--AAAATTATACTGT--GGGTGCGTGT---TACGCGTATCTGCAATCTTTGT-CA-- 2431
WTDNL15c6       ATA--AAAATTATACTGT--GGGTGCGTGT---TACGCGTATCTGCAATCTTTGT-CA-- 2427
WTDN15c9        ATA--AAAATTATACTGT--GGGTGCGTGT---TACGCGTATCTGCAATCTTTGT-CA-- 2426
WTDA21c4        ATA--AAAATTATACTGT--GGGTGCGTGT---TACGCGTATCTGCAATCTTTGT-CA-- 2433
WTDA21c5        ATA--AAAATTATACTGT--GGGTGCGTGT---TACGCGTATCTGCAATCTTTGT-CA-- 2432
WTDA21c6        ATA--AAAATTATACTGT--GGGTGCGTGT---TACGCGTATCTGCAATCTTTGT-CA-- 2458
Cow139c10       ATA--AAAATTATACTGTGTGGGTGCGTGT---TACGCGTATCTGCAATCTTTGT-CA-- 2451
Cow139c11       ATA--AAAATTATACTGTGTGGGTGCGTGT---TACGCGTATCTGCAATCTTTGT-CA-- 2453
Cow2c4          ATA--AAAATTATACTGTGTGGGTGCGTGT---TACGCGTATCTGCAATCTTTGT-CA-- 2453
Cow133c1        ATA--AAAATTATACTGTGTGGGTGCGTGT---TACGCGTATCTGCAATCTTTGT-CA-- 2452
Cow139c9        ATA--AAAATTATACTGTGTGGGTGCGTGT---TACGCGTATCTGCAATCTTTGT-CA-- 2452
Cow3535c3       ATA--AAAATTATACTGTGTGGGTGCGTGT---TACGCGTATCTGCAATCTTTGT-CA-- 2452
Cow3535c6       ATA--AAAATTATACTGTGTGGGTGCGTGT---TACGCGTATCTGCAATCTTTGT-CA-- 2452
Cow2c7          ATG--AAAATTATACTGTGTGGGTGCGTGT---TACGCGTATCTGCAATCTTTGT-CA-- 2451
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Cow2c10         ATA--AAAATTATACTGTGTGGGTGCGTGT---TACGCGTATCTGCAATCTTTGT-CA-- 2452
AB007814Tth     ATA--AAAATTATACTGTGTGGGTGCGTGT---TACGCGTATCTGCAATCTTTGTTCA-- 2453
Cow104c2        ATA--AAAATTATACTGT--GGGTGCGTGT---TACGCGTATCTGCAATCTTTGT-CA-- 2452
Cow104c3        ATA--AAAATTATACTGT--GGGTGCGTGT---TACGCGTATCTGCAATCTTTGT-CA-- 2452
Cow104c1        ATA--AAAATTATACTGT--GGGTGCGTGT---TACGCGTATCTGCAATCTTTGT-CA-- 2452
Cow2095c4       ATC--AAAATTATACTGT--GGGTGCGTGT---TACGCGTATCTGCAATCTTTGT-CA-- 2454
Cow2095c9       ATA--AAAATTATACTGT--GGGTGCGTGT---TACGCGTATCTGCAATCTTTGT-CA-- 2454
Cow2095c8       ATA--AAAATTATACTGT--GGGTGCGTGT---TACGCGTATCTGCAATCTTTGT-CA-- 2454
WTDA3c9.1       ATATAAAAATTATACTGT--GGGTGTGTGTGCAAATGCACATCTGCAACCTTTGTTCACA 2401
WTDA3c9.4       ATATAAAAATTATACTGT--GGGTGTGTGTGCAAATGCACATCTGCAACCTTTGTTCACA 2401
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